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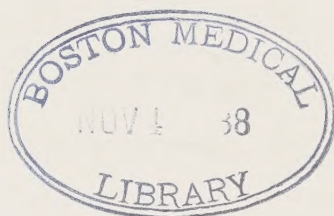
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# THE EFFECT OF ADMINISTERING FROG ANTERIOR PITUITARY SUBSTANCE TO IMMATURE FEMALE MICE <sup>1</sup>

A. ELIZABETH ADAMS AND GERTRUDE TUKEY

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FOUR PLATES (TWENTY-SIX FIGURES)

Reactions of the endocrine glands of animals to administration of anterior pituitary substance from individuals of a lower class than the recipient have seldom been reported. Stimulation of anuran ovaries with pars anterior from fish has been secured by Wills, Riley and Stubbs ('33) and Magdalena ('32) but negative results were obtained by Houssay, Giusti, Lascano-Gonzalez ('29), Rostand ('34) and Creaser and Gorbman ('36). By implanting pars anterior of frogs into the lizard, *Anolis*, Evans ('35) induced an ovarian reaction. Lipschutz, Kallas and Wilckens ('28) have provoked sexual maturity in infantile mice with implants of pars anterior from pigeons and Benoit ('35) obtained a similar effect using the anterior pituitaries of ducks that had been subjected to treatment with light for varying periods. His results with pituitaries from sexually inactive ducks were negative. More recently, Witschi, Stanley and Riley ('37) have stimulated the female reproductive system of immature rats and of hypophysectomized ones with injections of acetone-dried anterior pituitaries of turkeys.<sup>2</sup>

<sup>1</sup>Part of this work was reported at the meetings of the American Association of Anatomists, 1937. See *Anat. Rec.*, vol. 67, Suppl. no. 3, p. 2.

<sup>2</sup>Hogg ('37) has reported a stimulating action of the subneural gland of the ascidian, *Polycarpa tecta*, on ovaries of immature mice. He considers this reaction evidence of a 'pituitary function' for this gland.



The effects of implanting amphibian pituitaries into mammals have rarely been studied and the results have been consistently negative (table 1). In each case the number of pituitaries per animal and the number of tests were few.

TABLE 1  
*Results of administration of pars anterior of Anura to mammals*

| INVESTIGATOR                          | EXPERIMENT                                                                                                                                                                                                                                                     | RESULT                                   |
|---------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| Lipschütz and Paëz ('28)              | Pituitaries of Chilean frogs injected into immature mice and rats; 1 pit. daily for 7 days into 4 mice; 2 pits. daily for 6 days into 4 mice.                                                                                                                  | No effect on OVARY                       |
| Martins ('29)                         | Anterior lobes of Brazilian frogs ( <i>Leptodactylus</i> ) in full sexual maturity minced and grafted into infantile mice; (1) 2 A.L.; (2) 6 A.L. (2 each day for 3 days); (3) 2 A.L., 3 A.L., 4 A.L.; (4) 2 A.L., 1 A.L., 4 A.L.                              | No effect on OVARIES or UTERI            |
| Zavadovsky ('29)                      | "The activity of the frog's hypophysis has been shown to coincide qualitatively with that of mammals and birds." . . . "The hypophysis of the axolotl is considerably less active in comparison with other mammals." <i>Am. J. Physiol.</i> , vol. 90, p. 567. |                                          |
| Zondek ('31, '35)                     | One to 3 pituitaries of adult <i>Rana esculenta</i> transplanted into immature mice and rats.                                                                                                                                                                  | No effect on SEXUAL APPARATUS of host    |
| Magistris ('32)                       | Three tests for F.S.H. and L.H. with frog and two with toad pituitaries made on infantile mice.                                                                                                                                                                | No effect on OVARIES                     |
| Magdalena ('32)                       | Several dozens of anterior lobes of toads administered to guinea pigs.                                                                                                                                                                                         | Feeble or doubtful activation of THYROID |
| Houssay, Novelli and Sammartino ('32) | Hypophyses of normal or thyroidectomized toads (20 A.L.) administered to guinea pigs.                                                                                                                                                                          | Doubtful action on THYROID               |
| Magistris ('32)                       | Tests of pituitaries of cold-blooded animals (species?) into warm-blooded ones (species?)                                                                                                                                                                      | No FAT METABOLISM hormone                |

Moreover the investigations have dealt with the response of only one endocrine gland, usually the ovary or thyroid. In the present experiments, a much higher dosage of anuran anterior pituitaries was given and a larger number of animals (mice) was tested. Moreover, three endocrines were examined, ovaries, thyroids, adrenals. The ovaries appeared to give no response to implantations of the frog pituitaries in the amounts used, but the thyroid and adrenal glands showed a decided stimulation.

#### MATERIAL AND METHODS

The hosts were infantile albino mice, 18 to 19 or 21 to 22 days old at the beginning of treatment. The donors were frogs, *Rana pipiens*. With three exceptions females were used since Rugh ('34, '35 a, b, '37 a, b) has reported that the pars anterior of the female is about twice as potent as that of the male in gonad-stimulating hormone as judged by ovulation-inducing reaction. In all but three cases, fresh anterior pituitary substance was administered. In these three, acetone-dried powder was used. In each test, the host received a total of 16, 24, 32, 60, 64, 80, or 96 pars anteriors. Usually, one-fourth of the total number was administered each day for 4 days. When ninety-six pituitaries were implanted, the implants were made twice daily at 12-hour intervals, twelve glands being given each time. The pituitaries were weighed before being implanted and the number used for a single dose was macerated in 0.3 cc. of 0.9% salt solution before being injected. Litter mate controls received brain tissue in amounts approximately equivalent to the doses of pituitary. One litter was given acetone-dried pituitary powder from adult mice so that the response could be compared with those in the other series. Ninety-six to 100 hours after the first treatment, vaginal smears were taken and the animals weighed and killed. The ovaries, uteri and tubes, thyroids and adrenals were dissected out, weighed and fixed for histological study. The reproductive system was preserved in Bouin's fluid, the thyroid and adrenal glands in Helly's fluid. The ovaries were cut at  $10\mu$  and stained in Mayer's haematoxylin and eosin.

The thyroid and adrenal glands were cut at 5 or 10  $\mu$  and stained in Mallory's connective tissue stain or in Harrison's modification of Mallory's stain.

There were thirty-five experimental mice and nineteen controls. In the tables only thirty-one experimental and seventeen control mice are listed since in two cases there was some possibility that the animals were mixed at killing and in one litter copper was administered with the pars anterior in what proved to be toxic amounts. These animals died or were killed at 72 hours after the first treatment instead of 96 as was true of all the other tests.

Counts of ovarian follicles were made for a number of animals. In such counts, every section of the ovary was examined and the follicle counted only when the nucleus of the egg was seen.

The height of the thyroid epithelium was determined by projecting ( $\times 500$ ) the middle section of the glands of 18- to 19-day-old mice that had been given twenty-four and ninety-six anterior pituitaries. The heights of the most columnar and most squamous cell in each follicle were measured and averaged. The total number of follicles in these sections was also secured and the color of the colloid in each follicle was recorded.

In estimating the hypertrophy of the adrenal gland, the volumes of the cortex and medulla were obtained by projecting every other section of a serially sectioned gland at a magnification of 100 times. These tracings were measured with a polar planimeter and the volume of the whole gland, medulla and cortex calculated. To compare the size of the cells in the zona fasciculata of experimental and control animals, the average diameter of 100 fascicular cells was secured. The diameter was based on the average of two measurements made at a magnification of 1000 times.

#### OBSERVATIONS

Examination of the tabulated data (table 2) indicates that even with large amounts of frog anterior pituitary substance





the response of the ovaries and of the tubes and uteri was negative. None of the vaginae opened. This contrasts with the reaction after mouse anterior pituitary implants. On the other hand, the thyroid and adrenal glands increased significantly both in their absolute weights and in their absolute weights expressed as percentages of body weights. The increase of the latter for the thyroids ranged from 87% in the group that received sixteen pituitaries to 301% in the animal given a suspension of an acetone powder of sixty pars anterior from female frogs. It averaged 133% for all of the experimental animals. In one case (with male pituitaries) it was only 13% but in only two others was it less than 70%. Although the increases in adrenal weights are less marked than those of the thyroids, nevertheless they are very definite in all except those animals that received sixteen pars anterior. In the latter the increase (adrenal weight expressed as a percentage of body weight) was only 2%, but with 24 P.A., the increase was 55%; with 64 P.A., 49%; and with 96 P.A., 64%. The average increase for all of the thirty-one experimental mice compared with the seventeen controls was 29%. The response of the thyroids and adrenals to the implantation of mouse pituitary was relatively slight or negative as judged by weights. Again using the organ weight as percentage of body weight, the thyroid increase was 13% while the adrenals showed no increase at all.

These facts are also brought out in the histological study made of the various organs. The ovaries (figs. 1 to 6) of the experimental and control animals were strikingly similar in appearance. Counts were made of the follicles in each of three mice of the older age group which received 24 P.A. and in their controls and in each of three mice of the younger age group which received 96 P.A. The follicles were classed as primary, those having two or more layers of granulosa cells; vesicular, those showing an antrum folliculi or the beginning of one; and atretic. The last group was again subdivided into primary and vesicular types (Engle, '27). The first (type I) consisted of small primary follicles with no antrum folliculi

and showed degenerative changes of the ova, indicated by the presence of pseudo-maturation spindles or spindles and polar bodies or fragmentation of the ova. Type II possessed an antrum folliculi in which the stratum granulosum either was greatly degenerated leaving a naked ovum or had a normal number of cells bordering on an antrum containing cells with pycnotic nuclei. Ova with an intact granulosum often had fragmented nuclei or showed pseudo-maturation spindles.

The data secured in the above counts were as follows. In the three 21- to 22-day-old hosts with 24 P.A., there was an average of 232 follicles of which 18.5% were primary, 14.7% vesicular and 66.8% atretic. Their three litter mate controls gave an average of 247 follicles, of which 27.5% were primary, 15.0% vesicular and 57.5% atretic. In both control and experimental animals 84% of the atretic follicles were of type I (primary). In the 18- to 19-day-old hosts with 96 P.A., the average number of follicles was 261. Of these 28.7% were primary, 18.4% vesicular, and 52.9% atretic. The average number of follicles in the controls was less—197—but the percentages of each kind were very similar to those in the experimental mice, 23.4, 20.8, 55.8%, respectively. Again type I is more abundant in the atretic follicles, composing 61% of those in the experimental mice and 65% in the controls. No fully developed graafian follicles were present in either experimental or control animals. Although only a small number of counts was made, it reinforces the evidence of the lack of stimulation in the data on weights. In the animals given mouse pituitary, vesicular follicles including many graafian ones were numerous and atretic follicles were fewer in number.

The histological appearance of the uteri agreed with the negative uterine response shown by organ weights (table 2). Cuboidal epithelium, characteristic of the immature uterus (Smith and Engle, '27) occurred in all animals, both experimental and controls, except those that were stimulated with anterior pituitary from adult male mice. Uteri of these hosts were characterized by a very high columnar epithelium with many mitotic figures and thickening of the submucosal and muscular coats.



The histological responses of thyroid and adrenal glands to anterior pituitary substance from frogs are in marked contrast to that of the ovaries (compare also data of table 2). The stimulation of the thyroid is seen both in the hyperplasia and hypertrophy of the follicular cells, increased numbers of follicles, decreased amount of colloid and a change in its consistency and staining reaction (figs. 7 to 14). The height of the follicular epithelium was measured in the center section of the thyroid gland in three of the mice that received 24 P.A. and in three with 96 P.A. and in their respective controls. The cellular height of the normal gland averaged  $6.21 \mu$ ; that in the hypertrophied gland,  $10.24 \mu$ . This enlargement (an average of 64.9%) was as great in the animals receiving 24 P.A. (an average of 35 mg. of fresh pars anterior) as in those receiving 96 P.A. (an average of 104 mg. of fresh pars anterior). It was not as great following 16 P.A. Besides the difference in height of the thyroid cells of control and pituitary-treated animals, their general appearance was very dissimilar. The cells of the normal or control thyroid were low cuboidal and contained very few vacuoles filled with unstained colloid (figs. 8, 12). Mitoses were rare. The cells of stimulated glands were high cuboidal to columnar often with their apical ends bulging into the follicular lumen (figs. 10, 14). Folds of the epithelium, sometimes even obliterating the lumen, were prominent in the glands stimulated by thirty-two or more frog anterior pituitaries.

Still further evidence of increased thyroid activity following administration of frog pituitary was secured by an examination of the follicles and their colloid content and a comparison with the control glands. The follicles were more closely packed together and their vascularity was greater in stimulated glands. Furthermore, their total number in the median section of the gland had increased 23% for the six counted (three with 24 P.A.; three with 96 P.A.) over the number in the six controls. Likewise the state of the colloid was used as a criterion of increased activity since new colloid or that being resorbed typically stains blue and old colloid

orange in Mallory's connective tissue stain and it also changed from a smooth compact substance to a more fluid state. In control glands, the majority of the follicles contained deep orange, smooth colloid and in the six that were counted only 22.8% of those in the median section contained blue colloid. In the glands from animals that were given frog pituitary, blue or bluish yellow color was predominant and in the six counted, 84.3% of the follicles in the median section were of this hue. Phagocytes and blood cells which are typical of hypertrophied glands (Loeb and Hasselberg, '20) were present in some of the follicles of all of the more highly stimulated thyroids of mice that were given frog pituitaries (fig. 14). The lack of activation in thyroids of control mice, i.e., those receiving frog brain tissue, was also characteristic of the mice to which mouse anterior pituitary substance was administered.

Study of the adrenal glands reveals that their increase in weight following administration of frog anterior pituitary is due to the enlargement of the cortex. This is evident in the determinations of volume made with the polar planimeter. In the five mice receiving 24 P.A., the volume increases of the entire gland and of the cortex were 23 and 27% respectively; in the six with 96 P.A., 36 and 44%, respectively. A more significant comparison is one in which ratio of medulla to cortex (M: C) is considered. Here the figures are as follows: five mice with 24 P.A., 1:8.18; six mice with 96 P.A., 1:10.93; five controls, 1:6.25. Some difficulty is encountered in evaluating the last figures because right and left glands were not kept separate after fixation and so it is not certain on which of the two glands volume determinations were made. However, it is well known that the left adrenal of normal mice is larger than the right (Howard-Miller, '27; Hett, '28). This difference holds for the seventeen controls and thirty one experimental animals of this series, the percentages being nine and three, respectively. Furthermore, this difference in size lies in the cortex (Hett, '26, '28), the medullas of the two glands being similar. Therefore, even though in the present comparison it is not known whether the volumes are those of

right or left glands, it is significant that the average percentage increases of 31% and 75% in the ratio of volume of medulla to cortex of adrenal glands of mice receiving 24 and 96 P.A. over the controls is more than three and eight times, respectively, the difference found normally between weights of right and left glands (9%). Thus if volume is proportional to weight, the average increase of 55% in ratio of volume of medulla to volume of cortex for all the animals in which measurements were made is far in excess of any difference in the same ratio which might exist between right and left glands.

The increased size is located in the zona fasciculata of the cortex (figs. 15 to 26). When measurements were made of the cells of this zone, their average diameter in the six mice receiving 96 P.A. was found to be  $14.41\mu$  while that of their controls was  $9.46\mu$ . This represents an addition of 52.3% to cellular diameter. It is principally in the cytoplasm that this enlargement occurs, since the nuclei appear little changed (figs. 19 to 26). Furthermore in a normal adrenal gland the fascicular cells contain large discrete liquid droplets, while in the hypertrophied ones, the droplets are smaller and more numerous. Without special techniques for fixation this results in a lightly staining normal cell and a darkly staining hypertrophied one. Transition cells with discrete lipoid droplets around the nucleus and diffuse ones in the peripheral part of the cell were often found. Mitoses were frequent throughout the zona fasciculata and in the zona glomerulosa while in normal glands they are mainly confined to the outer fasciculata and the glomerulosa (Whitehead, '33).

The region between the zona fasciculata and the medulla has been variously described in the mouse as the zona reticulata (Tamura, '26) or the X-zone (Howard-Miller, '27; Deanesly, '28; Nurnberger, '32). In females it is typically present up to 80 days or even longer—200 days—in nulliparous individuals (Howard-Miller, '27) but pregnancy hastens its degeneration (Deanesly, '28; Masui and Tamura, '26; Takewaki, '36; Tamura, '26). This zone was present in both normal and stimulated glands. An area of lightly staining cells was found



next to the zona fasciculata in the adrenal glands of the two mice which received 64 P.A. and in five of the six which received 96 P.A. (figs. 16, 18). It is possible that this area represents a beginning of degeneration of the zona reticulata which characteristically occurs in the female mouse later in its life cycle.

#### DISCUSSION

It is evident from these experiments that frog anterior pituitary substance in the amounts used did not stimulate the ovaries and reproductive tract of immature female mice, but that their thyroid and adrenal glands underwent an enlargement that was significant in amount and that was evident in their histological appearance.

The explanation of the failure of the ovary to respond is difficult to determine. Other investigators (table 1) have reported similar results, but the doses of pituitary used were relatively small and the tests few. In the present experiments large amounts of anterior pituitary substance were administered ranging from an average of 16.2 mg. (16 P.A.) to 104 mg. of fresh pituitary (96 P.A.)<sup>3</sup> per animal (table 2). It would seem that this quantity should be adequate, especially when compared with the response of the mouse ovary to an average of 7.9 mg. (fresh weight) of mouse pituitary (administered in dried form) (table 2). However, Gallien ('37) has found that six to nine frog hypophyses (representing a few milligrams of substance) will induce ovulation in *Rana temporaria*, while 1.6 to 3 gm. (aqueous or alkaline extract) of fresh beef gland, i.e., roughly 200 times as much, are necessary to induce an equivalent reaction. Perhaps the numerous failures reported in the attempt to induce ovulation in anurans (frogs, toads) by mammalian implants or extracts (Adams, '31; Creaser and Gorbman, '36; Houssay and Giusti, '30; Houssay, Giusti and Lascano-Gonzalez, '29; Novelli, '32; Rostand, '34, '35; Rugh, '34, '35 a, b; Wills, Riley and Stubbs,

<sup>3</sup> From data secured recently on weights of fresh and dried pars anterior, this amount probably represents about 24.1 mg. of dried powder.

'33) have their explanation in the inadequate quantities used. Even within the mammalian groups marked variations in potency of anterior lobes exist. Loeb ('32 b) reports equivalent reactions on sex organs and thyroid of guinea pigs with 3 mg. of rat anterior lobe, 4 to 6 mg. of rabbit anterior lobe, and 44.5 mg. of guinea pig anterior lobe.

It is further possible that although pituitaries of *Rana pipiens* are potent during the interbreeding season judged by their ability to induce ovulation in amphibians (Wolf, '29; Rugh, '34, '35 a, b, '37 a, b) and in lizards (Evans, '35), this potency may not be as high as at some other part of the year. The histological appearance of the pars anterior of anurans in the spring (Zahl, '35; Stutinsky, '36) and the smaller dosage of anterior pituitary substance necessary to induce the ovulation reaction as the breeding season approaches (Rostand, '34; Rugh, '34, '35 a, b, '37 a, b) suggest that the potency per unit of gland may be higher in the spring than in the fall and winter. Martins ('29), however, used pituitaries from frogs in full sexual activity, but in relatively small amounts. This possibility must be tested.

It is also possible that some 'zoological specificity' of the hormones exists although this seems unlikely in view of the fact that the thyroid and adrenal glands reacted positively. Furthermore, such specificity does not seem characteristic of the hormones of the endocrine glands even when those of cold blooded animals are administered to warm-blooded ones (ovarian hormones in invertebrates, fish, frogs: Fellner, '25; Frank, '29; Loewe, Lange and Käer, '29; Steidle, '30; Loewe, Raudenbusch, Voss and Van Heurn, '32; Fleischmann and Kann, '32; thyroid hormones in reptilian thyroids: Swingle and Martin, '26).

One other suggestion should be mentioned. If the hyperplasia of the thyroid is indicative of a hyperthyroid condition in the mice that were given frog anterior pituitary—and a similar histology has been shown to go with increased metabolism in mammals (Verzár and Wahl, '31)—then this condition may have inhibited the reaction of the ovary. Such a possibility is suggested by the experiments of Loeb ('32 a) who

found that with increase in hypertrophy of the guinea pig thyroid through activation by anterior pituitary substance of the rat, the number of mature follicles decreased. Furthermore activation of the gonads of guinea pigs with various preparations of cattle anterior pituitary was hypotypical and ovulation was only obtained after long continued injections when the effect of the extracts on the thyroid activity had ceased. Fluhmann ('34) also showed that injections of gonad- and thyroid-stimulating extract of sheep pituitary were more effective in partially or completely thyroidectomized rats than in normal ones and that feeding of desiccated thyroid substance or administration of thyroxin to immature rats injected with acid gonad-stimulating extract of sheep anterior lobe caused lessened increase of uterine and ovarian weights. It is possible that the results of Smith and Engle ('27) who found increased ovarian weights in thyroidectomized rats given anterior pituitary implants indicate this relationship although they interpret them as probably due to the fact that the general body weights of the animals of this litter were above those of the usual litters.

As far as the thyroid and adrenal glands are concerned, the type of response to frog pituitaries is in no way abnormal. In both glands it resembles the conditions resulting from homoplastic, heteroplastic, xenoplastic, or dysplastic treatment.<sup>4</sup> For the thyroid this response is an increased weight associated with hypertrophy and hyperplasia of the follicular epithelium, a liquefaction and decrease in amount of stored colloid and an increase in vascularity of the gland.<sup>5</sup> For the adrenal, there is a weight increase and the hypertrophy is in the cortex, mainly in the zona fasciculata with a change in the distribution of the lipoidal content and an increase in numbers

<sup>4</sup>References are too abundant to cite. It is of particular interest that thyroids of birds, reptiles, amphibians and fish have been stimulated with mammalian anterior pituitary extracts; thyroids of amphibians with bird anterior pituitary; adrenals of amphibians with mammalian extracts; of snakes with snake implants; of mammals with mammalian extracts.

<sup>5</sup>See especially works of Aron, Loeb, Collip, Severinghaus, Houssay and their co-workers.



and size of its cells (Emery and Atwell, '33) and distribution of mitotic activity (compare Whitehead, '33). So it is seen that regardless of the species of the donor, the method of response of the end organ, when such is obtained, does not vary but follows a pattern for hypertrophy and hyperplasia. Thus the stimulation of the thyroid and adrenal of the mouse with frog pituitary substance, i.e., of dysplastic origin, is in agreement with the growing concept of the non-specificity of hormones between species.

The X-zone of the adrenal deserves further mention. No hastening of its degeneration seems evident except possibly in those mice that received 64 and 96 P.A. Here the zone seemed somewhat lighter in its staining reaction as if its disappearance had begun. Martin ('30) has reported no effect on the X-zone with injections of follicle-stimulating and luteinizing hormones of the anterior pituitary for 3 to 8 days. Nurnberger ('32) has found that it has a tendency to disappear with injections of pregnancy urine and Takewaki ('35) has discovered that six injections in 3 days of this substance had no effect on the zona reticulata but that treatment for 6 to 14 days (two injections daily) produced in some females complete or nearly complete degeneration of this zone. But this effect did not occur in castrated females and he therefore interprets it as an indirect action of the ovarian hormone. Since no such reaction occurred in the present experiments this might be considered as additional evidence of a lack of stimulation of the mouse ovary by the frog pituitary.

#### SUMMARY

1. Injections of saline suspensions of sixteen to ninety-six fresh or acetone-dried anterior pituitaries of frogs (*Rana pipiens*) were given daily for 4 days to twenty-five 18- to 19-day-old and six 21- to 22-day-old female mice and the hosts killed 96 to 100 hours after the first injection. Litter-mate mice received equal amounts of anuran brain tissue. One litter was given mouse anterior pituitary or brain for comparison.

2. No precocious sexual maturity, i.e., maturing of ovaries and of the reproductive tract, was induced in the mice by injections of frog pituitary, but homoplastic pituitary administration gave a positive response.

3. Hypertrophy and hyperplasia of the thyroid glands of mice were produced by the injections of frog pars anterior. A similar response did not occur with the mice pituitaries.

4. Treatment with twenty-four to ninety-six fresh frog anterior pituitaries caused hypertrophy of the adrenal cortex in mice. This was greater in those receiving sixty-four or more pituitaries than in those which were given fewer. Treatment with sixteen fresh glands or with a suspension of acetone-dried pituitary substance of twenty-four male frogs or of mice did not result in cortical hypertrophy.

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## PLATE 1

## EXPLANATION OF FIGURES

Photomicrographs of mouse ovaries.  $\times 37.5$ . Fixed in Bouin's fluid; stained in hematoxylin and eosin.

1 Ovary of control mouse, given frog brain tissue, 22 to 23 days old when killed.

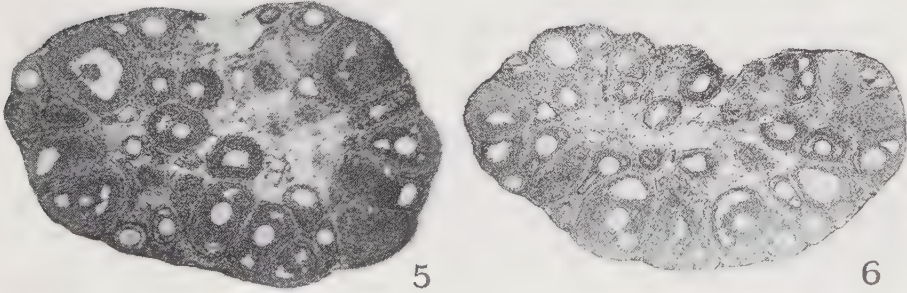
2 Ovary of mouse injected with saline suspension of 24 P.A. of female frogs in 4 days; 22 to 23 days old when killed. Litter mate of mouse of figure 1. No difference in ovaries is apparent.

3 Ovary of control mouse given frog brain tissue; 26 to 27 days old when killed.

4 Ovary of mouse injected with saline suspension of 24 fresh P.A. of female frogs in 4 days; 26 to 27 days old when killed. Litter mate of mouse of figure 3. No stimulation of ovaries has occurred.

5 Ovary of control mouse given frog brain tissue; 23 to 24 days old when killed.

6 Ovary of mouse injected with saline suspension of 96 fresh P.A. of female frogs in 4 days; 23 to 24 days old when killed. Litter mate of mouse of figure 5. No stimulation of ovaries has occurred.





## PLATE 2

### EXPLANATION OF FIGURES

Photomicrographs of thyroid glands of female mice, 22 to 23 days old when killed. Fixed in Helly's fluid; stained in Mallory's connective tissue stain.

7 Part of thyroid of control mouse given frog brain tissue.  $\times 160$ . Note low cuboidal epithelium and smoothly staining colloid.

8 Small section of thyroid of figure 7.  $\times 712.5$ .

9 Part of thyroid of mouse (litter mate of animal of fig. 7) given saline suspension of 24 fresh P.A. of female frogs in 4 days.  $\times 160$ . Note hypertrophy of cells and diminution in colloid. Compare figure 7.

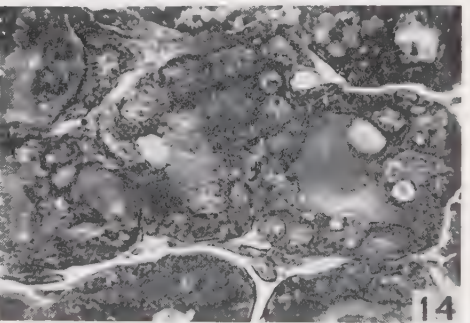
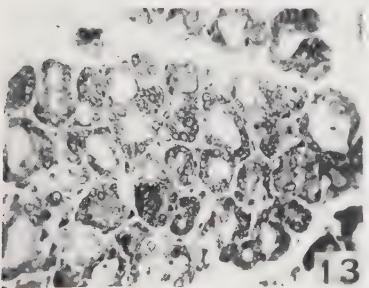
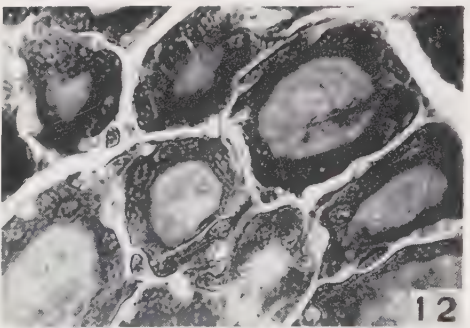
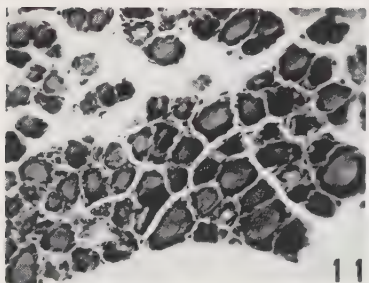
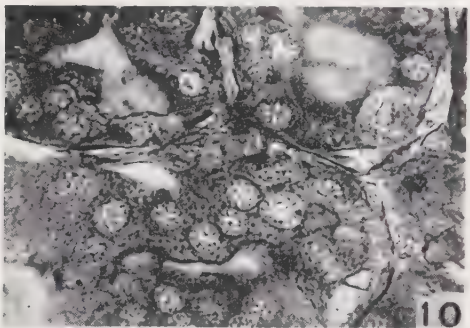
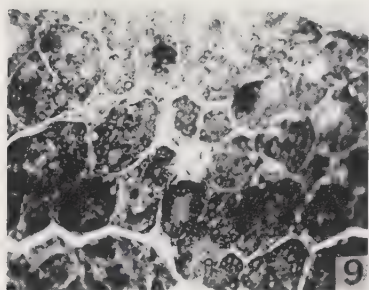
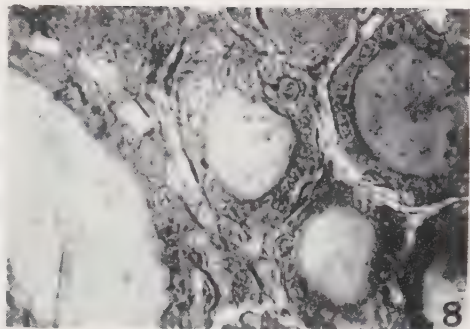
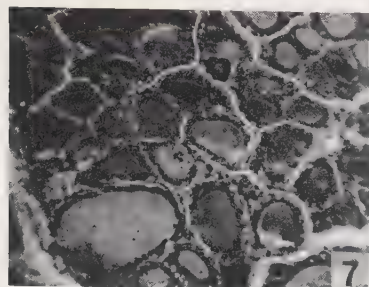
10 Small section of thyroid of figure 9.  $\times 712.5$ . Compare figure 8.

11 Part of thyroid of control mouse given frog brain tissue.  $\times 160$ . Note low cuboidal epithelium and smoothly staining colloid.

12 Small section of thyroid of figure 11.  $\times 712.5$ .

13 Part of thyroid of mouse (litter mate of animal of fig. 11) given a saline suspension of 96 fresh P.A. of female frogs in 4 days.  $\times 160$ . Note hypertrophy of cells and decreased colloid in follicles. Compare figure 11.

14 Small section of thyroid of figure 13.  $\times 712.5$ . Compare figure 12.



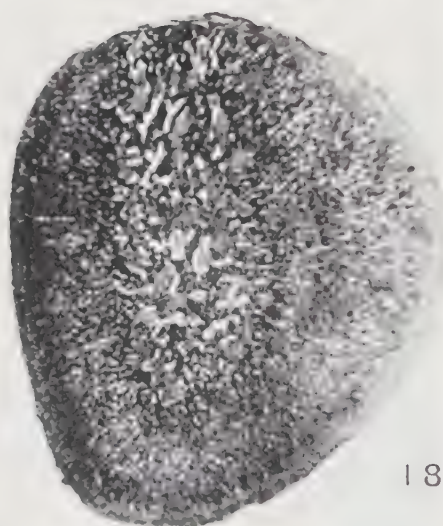
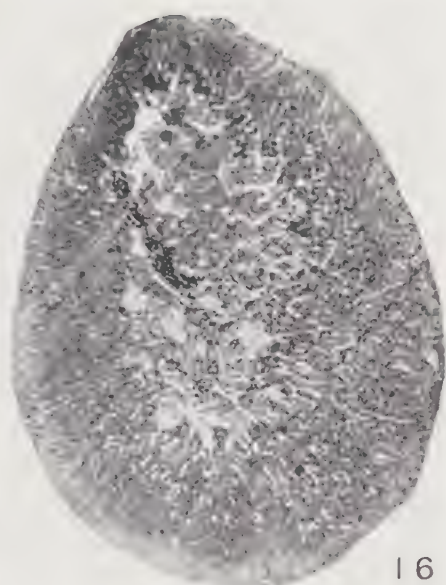
### PLATE 3

#### EXPLANATION OF FIGURES

Photomicrographs of adrenal glands of female mice 22 to 23 days old when killed.  $\times 50$ . Fixed in Helly's fluid; stained in Mallory's connective tissue stain.

- 15 Adrenal of control mouse given frog brain tissue.
- 16 Adrenal of mouse injected with saline suspension of 24 fresh P.A. of female frogs in 4 days. Litter mate of animal of figure 15.
- 17 Adrenal of control mouse given frog brain tissue.
- 18 Adrenal of mouse injected with saline suspension of 96 fresh P.A. of female frogs in 4 days. Litter mate of animal of figure 17.





## PLATE 4

### EXPLANATION OF FIGURES

Photomicrographs of adrenal glands of female mice, 22 to 23 days old when killed. Fixed in Helly's fluid; stained in Mallory's connective tissue stain.

19 Section of cortex of adrenal of control mouse (see fig. 15).  $\times 160$ .

20 Detail of fascicular cells of cortex of figures 15 and 19.  $\times 712.5$ . Note small tightly packed cells and cytoplasm with large vacuoles resulting from dissolution of lipid droplets.

21 Section of cortex of adrenal of mouse (litter mate of animal of figs. 16 and 19) given a saline suspension of 24 fresh P.A. of female frogs in 4 days.  $\times 160$ .

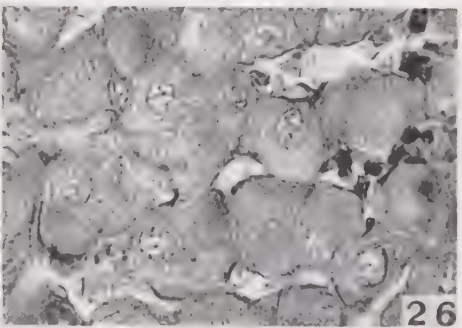
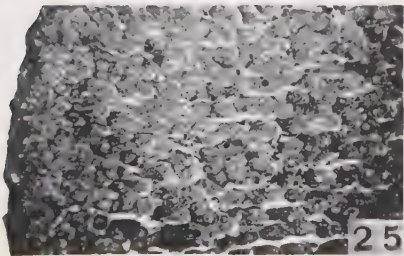
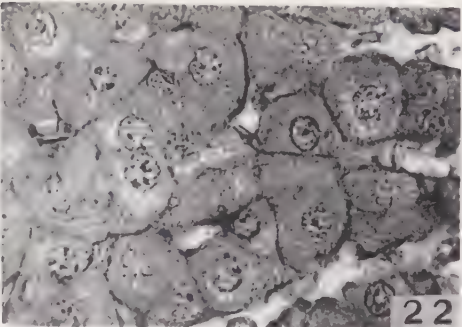
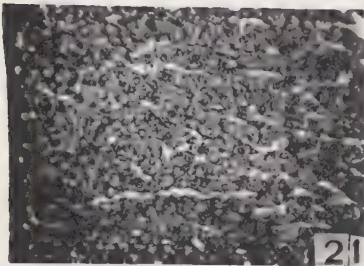
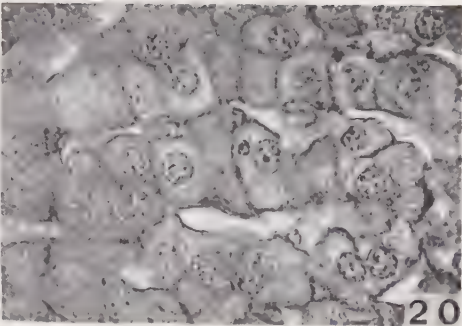
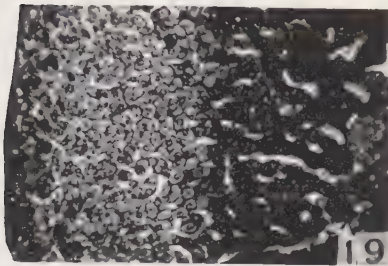
22 Detail of fascicular cells of cortex of figures 16 and 21.  $\times 712.5$ . Note increased amount of cytoplasm of cells which contains numerous small vacuoles resulting from dissolution of lipid droplets.

23 Section of cortex of adrenal gland of control mouse (see fig. 17).  $\times 160$ .

24 Detail of fascicular cells of cortex of figure 23.  $\times 712.5$ . Note small tightly packed cells.

25 Section of cortex of adrenal of mouse (litter mate of animal of figs. 17 and 23) given a saline suspension of 96 fresh P.A. of female frogs in 4 days.  $\times 160$ .

26 Detail of fascicular cells of cortex of figure 25.  $\times 712.5$ . Note very large amount of cytoplasm containing small vacuoles as result of dissolution of lipid droplets.







# A NOTE ON THE HISTOLOGY AND PHARMACOLOGY OF THE HYPOPHYSIS OF THE MANATEE (TRICHECHUS INUNGUIS)

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## TWO FIGURES

Through the cooperation of Dr. W. H. Chute, director of the Shedd Aquarium of Chicago, we were afforded the opportunity of performing an autopsy on a young South American male manatee (*Trichechus inunguis*), the only specimen to our knowledge that was in captivity in this country. The fact that we were unable to find in the literature any description of the hypophysis of this species seemed sufficient justification for publishing this note.

The pituitary gland was removed along with some adhering connective tissue and divided sagittally into two approximately equal parts, one of which was placed in acetone, the other was fixed in formol-Zenker. After fixation, this portion was embedded in celloidin and serially sectioned at 8  $\mu$ . The mounted sections were then stained with hematoxylin-eosin-azure and the Mallory-azan stains. Despite the fact that some 5 hours had elapsed between the death of the animal and the fixation of the tissue, the preservation was found to be fairly good.

The gland consists of two lobes separated by a thick layer of dense connective tissue. The neural lobe is incompletely subdivided by prominent strands of connective tissue containing numerous blood vessels. Nuclei of pituicytes are seen in fair abundance though Herring bodies are relatively scarce. There is no evidence of any envelopment or interdigitation of the neural lobe by pars intermedia cells. Furthermore, gross or microscopic evidence of an intermediate lobe is lacking.

The anterior lobe is divided by thin strands of connective tissue into lobules in which the cells are arranged in closely packed cords. Four main types of cells and many intermediate forms can be distinguished. Of these, acidophils and chromophobes are in general similar to those found in other species. The cytoplasm of the chromophobes is scant and stains palely. The other two types of cells are basophilic in their staining properties. In one, the cytoplasm appears slate-colored in the Mallory-azan preparations. The nucleus is large and vesicular and contains one or more prominent

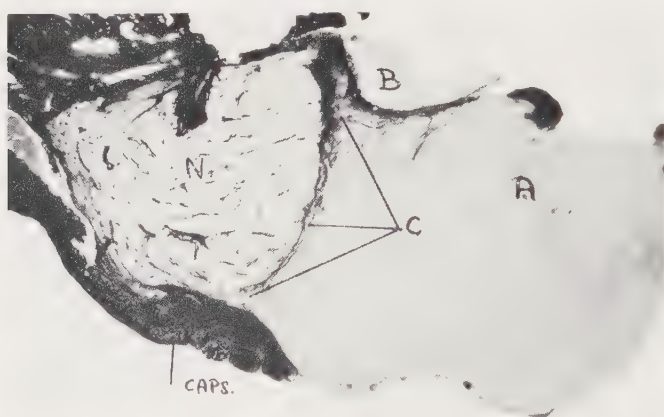


Fig.1 Low power field of manatee hypophysis  $\times 13.2$ . N, neural lobe; A, anterior lobe; C, cleft; B, brain; Caps., capsule.

nucleoli and a sprinkling of chromatin. The other type is much less numerous and with the above stain, the cytoplasm is an intense blue. A graded series of cells can be traced between these two types of basophils and the chromophobes on one hand and the acidophils on the other. Since fixation was delayed, it is impossible to determine whether the slate-colored cells are true basophils or one of the other cell types in which the cytoplasm has undergone post mortem changes. For the same reason, it is impossible to determine the significance of a colloid degeneration that is frequently found in the chromophil cells. This degeneration varies in intensity

from a few drops to a condition in which practically the entire cytoplasm becomes a colloid mass.

The arrangement of the cells is on the whole rather haphazard. The acidophils are more numerous at the periphery of the lobe while in its anterior portion, there is a fairly extensive and well-circumscribed area which consists almost entirely of the large pale-staining basophils. The chromophobes are more numerous in the center of the individual lobules.

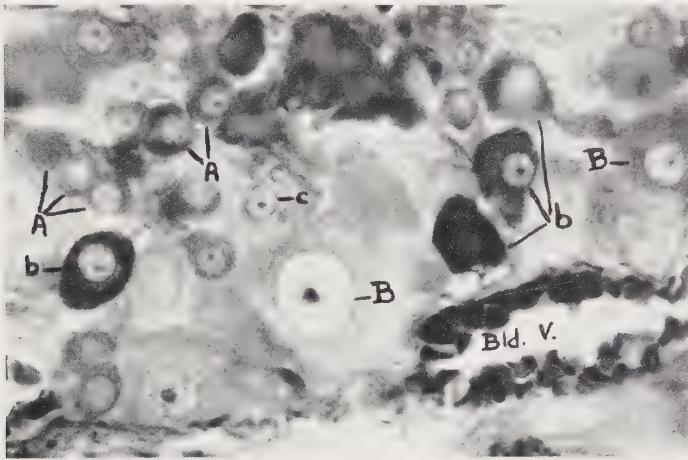


Fig.2 High power field showing various cell types.  $\times 725$ . A, acidophilic cell; B, large pale staining basophilic cell; b, dark staining basophilic cell; c, chromophobe; Bld.v., blood vessel.

A large cleft, lined with low columnar and flattened non-granular epithelium extends along the entire posterior margin of the anterior lobe. It is separated by a single layer of cells only from the connective tissue partition between the two lobes. This cleft, which is presumably a remnant of Rathke's pouch, sends off branches into the anterior lobe and short extensions into the connective tissue septum. In no place, however, do the epithelial cells invade the neural lobe. According to Wislocki ('29) and Wislocki and Geiling ('36) a residual cleft is not present in the hypophyses of the porpoise

and the whale. We have recently sectioned the hypophysis of a finback whale fetus of approximately  $2\frac{1}{2}$  months and even at this early stage no cleft is visible though the extension of the third ventricle into the neural lobe is still patent. The pituitary of the manatee therefore differs from that of the above-named aquatic mammals in that this lumen persists at least in the young adults. It is possible that the cells lining the cleft represent the intermediate lobe but proof of this must await the development of a differential stain for intermediate lobe cells.

Along the anterior margin of the cleft, cells of the anterior lobe appear in places to be breaking through the epithelial lining while the lumen contains much cellular debris, among which chromophobes and red blood cells can be recognized. It is probable that most of these cells entered as the result of unavoidable injury during the removal of the gland, the removal being complicated by the fact that the skull was to be mounted. The minimal amount of cutting was therefore possible and the gland had to be removed through a small enlargement of the foramen magnum.

Toward the extreme anterior margin of the lobe, there are several cyst-like structures lined with low epithelium and filled with a blue-staining colloid material. Such structures are commonly encountered in the hypophyses of other species. Their origin and significance is at present an open question.

Non-granular epithelial cells are also seen to underlie a small portion of the brain which was removed with the stalk. These cells are separated from the brain by the very vascular leptomeninges and from the rest of the gland by the dura and represent undoubtedly a portion of the tuberalis.

The meningeal relationships were unfortunately not complete. The gland is embedded in a closely adherent connective tissue capsule, the dura. This however does not show the marked vascularity that has been described in the capsule surrounding the hypophysis of the whale (Wislocki and Geiling, '36).



The portion of the gland fixed in acetone was stripped of its connective tissue and the two lobes separated with comparative ease along the septum. They were ground up separately and extracted with acetic acid according to the specifications set forth in the United States Pharmacopoeia XI for the preparation of standard pituitary solution. The two extracts were then compared with a standard preparation for their oxytocic, pressor, antidiuretic and melanophore-expanding activities by the standard methods. Since the amount of material was limited, complete quantitative assays could not be made. However, the results were adequate and sufficiently consistent to warrant our conclusions.

The oxytocic activity of the posterior lobe was found to be about 50% of the standard powder while that of the anterior lobe powder was less than 10%. This small amount is in all probability due to contamination. The pressor activity of the posterior lobe was found to be about 50% of the standard. The antidiuretic activity was present in the posterior lobe. However, extracts from this lobe showed only at best a doubtful trace of melanophore-expanding activity. In contrast, the equivalent of 1 mg. of anterior lobe powder had a very marked melanophore-expanding effect in the frog, the animal remaining dark for over 5 hours. Thus the neural lobe of the manatee hypophysis contains oxytocic, pressor and antidiuretic but no melanophore-expanding activity. This latter property is found in the anterior lobe extracts.

It is generally assumed that the melanophore-dilating hormone arises from the intermediate lobe which in most species is closely applied to and virtually inseparable from the posterior lobe and which is therefore included in the posterior lobe extracts. Since no *pars intermedia* could be demonstrated in relation to the posterior lobe of the manatee hypophysis, and since a dense connective tissue septum was shown to separate the neural from the epithelial lobe, one would not expect to find melanophore-dilating activity in the neural lobe extracts. In this respect, the gland of the manatee is similar to that of certain other species such as the whale

(Geiling, '35), chicken (DeLawder, Tarr and Geiling, '34) and armadillo (Oldham, '38).

#### SUMMARY

The hypophysis of the manatee consists of an anterior and a neural lobe separated by a dense connective tissue septum. Histological evidence of an intermediate lobe is lacking.

Extracts of the neural lobe contain oxytocic, pressor and antidiuretic activities. The melanophore-expanding hormone is present, not in the neural, but in the anterior lobe.

The absence of epithelial cells invading the neural lobe and the complete anatomical separation of the two lobes affords additional proof that the posterior lobe hormones are formed by intrinsic elements of the posterior lobe (Geiling and Oldham, '37).

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# STUDIES OF THE ISLANDS OF LANGERHANS AFTER CONTINUOUS INTRAVENOUS INJECTION OF DEXTROSE<sup>1, 2</sup>

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THREE TEXT FIGURES AND THREE PLATES (ELEVEN FIGURES)

## INTRODUCTION

These experiments were undertaken to determine the effect of continued elevation of the blood sugar on the islands of Langerhans in the intact pancreas. Homans ('15) and Allen ('22) working independently, studied the islands of Langerhans after the removal of a sufficient amount of the pancreas in dogs and cats to produce a continuous glycosuria on a diet of bread and meat. They both found hydropic degeneration in the cells of the islands, particularly the beta cells: this was taken as an indication that continuous hyperglycemia would produce the hydropic degeneration by overwork and fatigue of the beta cells, and, thereby, establish a true diabetes. If this were the most important factor it would seem that continuous injection of large amounts of dextrose might produce similar changes in the intact pancreas.

Woodyatt ('15), who first designed an apparatus for continuous intravenous injection, injected into dogs large quantities of dextrose. He found that quantities greater than about 0.9 gm. per kilogram per hour would produce glycosuria

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<sup>2</sup> This work was done under the direction of Dr. Sylvia H. Bensley. Submitted in partial fulfillment of the requirements of The University of Chicago for the degree of Doctor of Philosophy.

within 30 minutes; that the amount of dextrose necessary to produce glycosuria was approximately twice the amount consumed in producing the total heat lost under the conditions of his experiments; that there was a marked increase in metabolic rate, but that this increase could account for but a small part of the total amount of dextrose injected; and that there seemed to be a rather definite maximum above which the total metabolic rate could not be increased by the injection of larger quantities of dextrose. Jacobs and Colwell ('36) studied blood chemistry during the continuous injection of as much as 4.25 gm. of dextrose per kilogram per hour, injected as long as 6 days.

Since in the experiments of Woodyatt ('15) and Jacobs and Colwell ('36) the continuous injection of dextrose was maintained for no longer than 6 days with no resulting diabetes, and no cytological studies were made of the islands of Langerhans, it seemed worthwhile to study the pancreas by the cytological methods developed by Bensley ('11), after the prolonged injection of various amounts of dextrose.

#### MATERIALS AND METHODS

Forty apparently healthy male guinea pigs weighing between 360 and 840 gm. were injected continuously with a 30% solution of dextrose to which had been added 0.75% of sodium chloride. As controls, two animals were injected with 2 cc. of 0.75% solution of sodium chloride per hour, one for 7 days and the other for 14 days.

Under ether anaesthesia, with aseptic technique, an incision was made in the neck of the animal and a small rubber tube, as described by Jacobs ('33), was put into the external jugular vein through a large hypodermic needle so that about 2 cm. of the tube were in the vein. The other end of the tube was brought out through the incision and the incision was closed. The small rubber tube was connected through a special glass cannula (fig. 2) (attached to the neck of the animal) with a larger rubber tube that went to a 50 cc. syringe held in an apparatus driven by a synchronous motor so that exactly



2, 3 or 4 cc. of the solution were injected during each hour, that was, between 0.71 and 2.45 gm. of dextrose per kilogram of body weight per hour.

The continuous injection apparatus consisted of a device into which from one to three 50 cc. syringes were clamped and arranged so that the plungers of the syringes were pushed by means of a synchronous motor, which assured a constant rate (fig. 1). The syringes, the tubing, the bottle containing the solution, and the glass cannula were wrapped in a towel and autoclaved. The apparatus was so designed that the syringes and the connecting tubing would not have to be taken apart in order to connect them with the apparatus.

In order to make the conditions of the experiment as nearly normal as possible, the animals were kept in cages rather than tied down to a board. It was found necessary to make the cages narrow enough so that the animals could not turn around, about twice the length of the animals and just high enough so that the animals could stand, move forward and backward comfortably and yet could not climb up on the side of the cage and turn over. In the top of the cage was a slit through which the tubing passed (fig. 3). (If there is enough room in the cage the animals will invariably turn around and twist the tubing so that the injection will be stopped, and if they can reach it, they will chew the tubing and make it leak.)

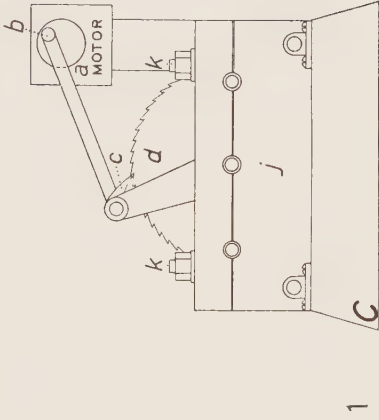
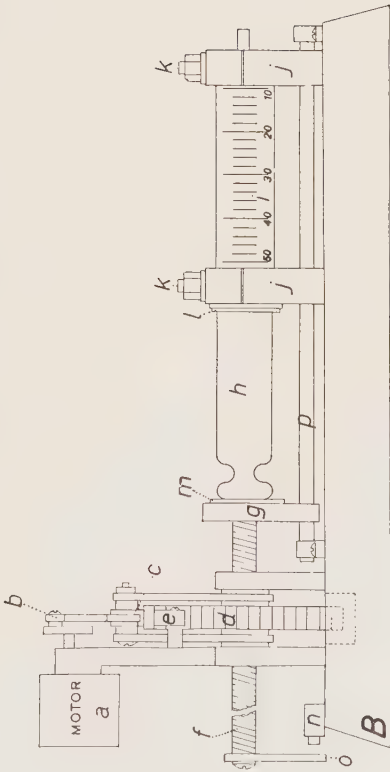
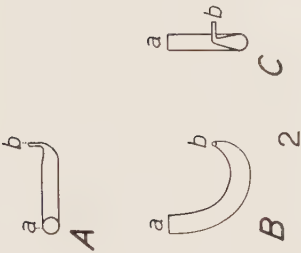
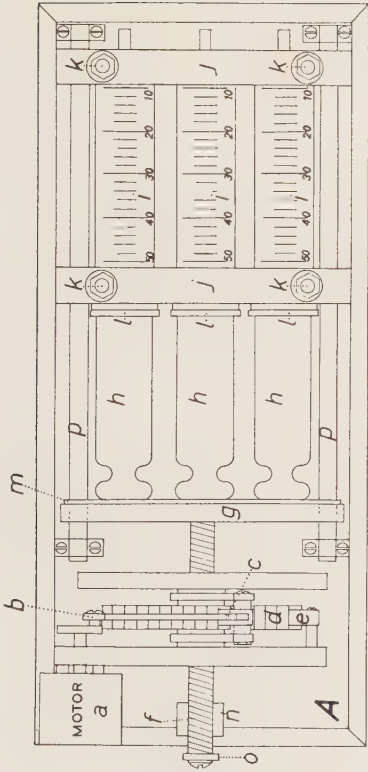
At the end of each experiment the tissue was fixed in formalin-Zenker (for adequate preservation of secretion granules), Bensley's AOB, and Regaud (for adequate preservation of mitochondria). Pieces of the livers were fixed in formalin-alcohol for glycogen studies. In a few of these experiments the animals were perfused with Janus green by Bensley's method, fixed with ammonium molybdate, dehydrated in absolute alcohol, cleared with benzol, and mounted in balsam. In dehydrating and clearing these tissues it was found necessary to cut the pancreas into three or four pieces as this considerably facilitated the dehydration and clearing. After clearing, the benzol was poured off and balsam poured in and the vessel containing the tissue was placed under a

bell jar that was evacuated. This facilitated the evaporation of the benzol so that when the tissue was mounted between glass plates the balsam was more easily hardened. These preparations were then bound with lantern slide tape. If adequate illumination is available, these can be projected and the islands of Langerhans show up very well (figs. 11, 12, 13 and 14). The tissue fixed in formalin-Zenker was stained with neutral gentian and the tissue fixed in Bensley's AOB was stained with aniline acid fuchsin and methyl green (Bensley, '11).

In one experiment about 80% of the pancreas was removed 7 days before injection was started. At the end of 7 days of injection the animal was perfused with Janus green. A piece of the remnant of the pancreas was fixed in formalin-Zenker. The larger part of the pancreas was fixed with molybdate and made into a whole mount. The tissue fixed in formalin-Zenker was embedded in paraffin and sections of it were stained with neutral gentian.

Fig. 1 The continuous injection apparatus. *A*, viewed from above; *B*, viewed from the side; *C*, viewed from the delivering end. *a*, synchronous motor that drives the apparatus at a constant rate. The shaft of the motor rotates at 2 r.p.m. *b*, eccentric on the shaft of the motor. The position of the eccentric can be changed so that the rate of injection can be changed. *c*, dog, that is activated by the eccentric (*b*). *d*, ratchet gear activated by the dog (*c*). *e*, a second dog that keeps the ratchet gear (*d*) from reversing. *f*, screw mounted in the center of the ratchet gear (*d*). *g*, bar driven by screw (*f*) when the ratchet gear (*d*) is rotated. *h*, plunger of 50 cc. syringe driven by the bar (*g*). *i*, 50 cc. syringe. *j*, split wooden blocks that hold the 50 cc. syringes in place. *k*, bolts that hold the blocks (*j*) together. *l*, rubber washers that protect the syringes from breakage. *m*, thin rubber pad that protects the plungers of the syringes. *n*, safety switch. *o*, metal strip placed on the end of the screw (*f*) to contact the safety switch (*n*). The length of the screw is just enough so that the switch will turn off the motor and prevent breaking the syringes should one fail to reset the apparatus. *p*, guide rods that keep the bar (*g*) aligned so that the plungers of the syringes are driven together.

Fig. 2 The special glass cannula. *A*, viewed from above; *B*, viewed from the side; *C*, viewed from the front. *a*, the larger end of the cannula that is connected to the rubber tubing leading from the continuous injection apparatus. *b*, the smaller end of the cannula that has been drawn small enough to pass into the special rubber tubing, after the special rubber tubing has been put into the external jugular vein of the animal. The cannula has been bent in three dimensions so that it fits closely to the neck of the animal.



In several of these experiments samples of blood were taken from the heart at different periods and the amount of reducing sugar determined by the Somgyi modification of

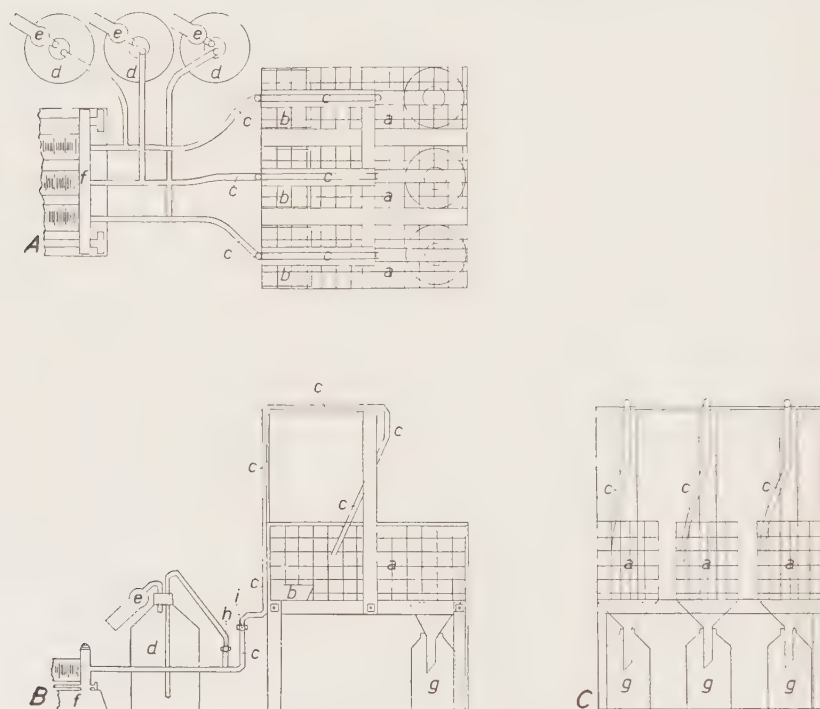


Fig. 3 The cages. *A*, viewed from above; *B*, viewed from side; *C*, viewed from end. *a*, cage in which guinea pig is confined. *b*, metal tray in which food is placed. *c*, glass and rubber tubing leading from injection apparatus to the animal. *d*, bottle containing 30% dextrose solution to which has been added 0.75% of sodium chloride. *e*, calcium chloride tube filled with cotton to prevent contamination of solution. *f*, the continuous injection apparatus. *g*, bottle in which the urine is collected for analysis. *h*, clasp on tube leading to bottle when machine is injecting. *i*, clasp on tube leading to animal that is closed when syringes are being refilled from the bottle (*d*).

the Shaffer-Hartman method as described by Peters and van Slyke ('32). Samples of the urine were tested by the same method. These determinations are summarized in the following table.



*Summary of data on experimental animals discussed*

| NUMBER OF DAYS | NUMBER OF ANIMAL | FIRST WEIGHT (GRAMS) | INJECTION (GRAMS/KILO PER HOUR) | NUTRI-TION | BLOOD SUGAR (MG./100 CC.)                                                                                                              |
|----------------|------------------|----------------------|---------------------------------|------------|----------------------------------------------------------------------------------------------------------------------------------------|
| 1              | 4                | 430                  | 1.39                            | Fair       | —                                                                                                                                      |
| 4              | 25               | 500                  | 1.20                            | Fair       | —                                                                                                                                      |
| 4              | 28               | 550                  | 1.00                            | Fair       | —                                                                                                                                      |
| 5              | 11               | 450                  | 1.33                            | Fair       | —                                                                                                                                      |
| 5              | 14               | 360                  | 1.66                            | Fair       | —                                                                                                                                      |
| 6              | 9                | 780                  | 0.76                            | Fair       | 6th day— 270                                                                                                                           |
| 6              | 12               | 440                  | 1.37                            | Poor       | —                                                                                                                                      |
| 7              | 17               | 600                  | 1.00                            | Fair       | —                                                                                                                                      |
| 16             | 19               | 840                  | 0.71                            | Fair       | Before starting injection— 109<br>16th day— 147<br>One hour later on 16th day<br>after injection stopped— 107                          |
| 28             | 20               | 780                  | 0.76                            |            | Before starting injection— 87<br>48 hours— 140<br>10th day— 147<br>11th day— 126<br>Injection stopped:<br>20 min.— 113<br>60 min.— 105 |
| 10             | 29               | 600                  | 2.00                            | Fair       | —                                                                                                                                      |
| 12             | 24               | 440                  | 2.25                            | Fair       | Before starting injection— 107                                                                                                         |
| 7              | 26               | 480                  | 1.00                            | Fair       |                                                                                                                                        |
| 7 <sup>1</sup> | 27               | 520                  | 1.00                            | Fair       |                                                                                                                                        |

<sup>1</sup> One week after removal of 80% of the pancreas.

## DESCRIPTION OF MATERIALS

Of the forty animals which were injected the following have been chosen for cytological description because of adequate fixation and staining and because they appear to be characteristic of the stages which they represent.

*Animal no. 4, injected 1 day.* The islands are of moderate size and stain palely. The beta cells are small, closely packed and contain few granules. The alpha cells are not numerous. There is no vacuolation in the acinar cells (figs. 5 and 5a).

*Animal no. 28, injected 4 days.* There is apparently a large amount of island tissue. The beta cells are small and well

filled with large discrete granules. The alpha cells are not numerous. A few of them stain poorly and are somewhat vacuolated. The acinar cells are poor in granules but not vacuolated.

There are a number of small islands consisting of a few beta cells, and individual beta cells are found within some acini. That these cells are true beta cells and not degenerating cells contain Mankowski granules may be determined by the size and staining of the granules. These may be compared in the same section, for a few Mankowski cells do occur. The granules in these intra-acinar beta cells are small, in some cases near the limit of visibility, while the Mankowski granules are larger, about halfway in size between the zymogen and beta granules. The beta granules stain a pale blue, whereas the color of the Mankowski granules approaches the gentian of the zymogen granules.

Occasionally a single cell containing both beta granules and zymogen granules may be found within an acinous (figs. 6 and 6 a). That these two types of granules occur within the same cell was determined by the examination of adjacent serial sections. They were studied in cells in which both types of granules are in focus in the same plane as the nucleus. These cells usually line the terminal portion of the lumen of the acinous, containing 1) less chromophile material than the adjacent acinar cells, and 2) granules that are identified as zymogen granules by their large size, intense stain, and position next to the lumen. The mitochondria are shorter than those of the typical acinar cells, but not as rod-like as those in the typical beta cells. These single cells are usually adjacent to a dilated capillary and contain specifically stained beta granules in the base of the cell.

Another animal (no. 25) injected for the same period, died some time before the pancreas was removed. Although there is some evidence of postmortem change, several mitoses occur in beta cells in many of the larger islands. The alpha cells are almost uniformly fragmented.

*Animal no. 11, injected 5 days.* There is apparently a large amount of island tissue. Occasional cells, identified as intercalated duct cells by their size, shape and position, contain specifically staining beta granules. In some of the acini near small islands individual cells with beta granules are found among cells containing zymogen granules (figs. 7 and 7 a). These cells were identified by the same characteristics which are exhibited by newly formed beta cells in the pancreas of the preceding animal, no. 28. That these cells are not a part of larger islands inserted between the acini was determined again by examining adjacent serial sections.

The larger islands stain darkly but some of them appear patchy due to the presence of alpha cells and large and small beta cells poor in granules. Most of the beta cells contain large discrete granules grouped at the poles of the cells.

The acinar cells are poor in granules and exhibit a moderate amount of vacuolation of the cytoplasm. The osmic acid preparations suggest that these vacuoles represent swollen and fatty mitochondria.

In another animal (no. 14) injected for the same period of time, the findings are essentially the same.

*Animal no. 9, injected 6 days.* The beta cells are of two types, one large and elongated with a polar distribution of the granules, and another small and polygonal with a variable granule content.

The alpha cells are not numerous and stain paler than normally. The mitochondria in the acinar cells are moderately swollen.

Another animal (no. 12) injected with almost twice as much dextrose for the same period of time exhibited essentially the same picture except that the changes in the alpha and acinar cells were more marked.

*Animal no. 17, injected 7 days.* The islands stain darkly but have a punched-out appearance. The light areas consist of alpha cells and other cells which have many of the characteristics of beta cells that have lost their granules, but are very difficult to differentiate from alpha cells. A few of

these pale staining cells exhibit hydropic degeneration (figs. 8 and 8 a). Many of the small beta cells are rich in large discrete granules. In the acinar cells the mitochondria are swollen and fatty (figs. 9 and 9 a).

*Animal no. 20, injected 28 days.* The islands stain darkly but are patchy due to the presence of many beta cells that are large, poor in granules and resemble alpha cells. The alpha cells are not numerous and appear pale except for a dark area close to the nucleus which stains with the nuclear dye (figs. 10 and 10 a). There is no vacuolation in the acinar cells and the mitochondria are normal.

*Animal no. 29, injected 10 days with 4 cc. per hour.* The beta cells are uniformly filled with large granules. The alpha cells are not numerous but stain normally. The mitochondria in the acinar cells are normal.

*Animal no. 24, injected 12 days with 3 cc. per hour.* The islands stain dark but are patchy due to alpha and beta cells which are poor in granules. Beginning hydropic degeneration is present in some of the alpha cells and in duct cells associated with the islands. The mitochondria in the acinar cells are moderately swollen.

*Animal no. 26, injected 7 days and perfused with Janus green.* The number of islands does not appear to be much more than that found in some normal animals (fig. 13). However, it should be noted: 1) that the larger islands are larger than in the normal 2) that the staining is more diffuse in many of the larger islands and 3) that there is considerable peripheral island formation in relation to acini, the so-called 'acinar type' of island. This would suggest that merely counting the number of islands is not adequate to demonstrate an increase in island tissue. But the perfusion method does give us a reliable technique not only for determining the total number of islands in the pancreas but also for visualizing changes in size and character of the islands throughout the pancreas, since it agrees with the histological findings.



Sections of a piece of this tissue fixed (after perfusion) in formalin-Zenker confirmed histologically the findings from perfusion.

*Animal no. 27, injected 7 days, after partial pancreatectomy; perfused with Janus green.* The preparations of the remaining piece of pancreas show an unusually large amount of island tissue (fig. 14). In sections of a piece of this tissue fixed (after perfusion) in formalin-Zenker there is considerable evidence of new island formation. There are many very small islands of the 'acinar type' and several mitoses occur in the islands near large ducts.

#### DISCUSSION

In these experiments the quantity of solution injected during 24 hours was about 10% of the body weight of the animal. The volume was thus restricted lest excessive amounts of fluid cause the animal to become markedly abnormal. In some of the experimental animals and in two control animals injected with physiological salt solution there developed a moderate amount of edema. The smallest amount of dextrose injected was 0.71 gm. per kilogram per hour in an animal that was injected for 16 days. The largest amount that was injected without increasing the volume of fluid was 1.66 gm. per kilogram per hour. Another animal that was injected with twice the volume of fluid received 2 gm. per kilogram per hour. The amount of dextrose injected may be estimated as approximately 150 to 500 mg. of sugar per 100 cc. of blood per minute. The increase in blood sugar was approximately only 50 mg. per 100 cc., which is very similar to that which was found in the experiments of Jacobs and Colwell ('36). In only one of the animals of this series was any appreciable amount of sugar excreted in the urine.

The animals receiving dextrose ate poorly, but that this loss of appetite was not due to the operative procedure was indicated by the fact that other animals, injected similarly with physiological saline or with insulin, ate very well. There was a loss of weight amounting to 20 to 30% of the original weight of the animal.

The absence of extensive 1) hyperglycemia, 2) glycosuria, or 3) storage of glycogen in the liver or muscles studied, but a marked loss of body weight seems to indicate a rapid oxidation of the sugar and an increase in basal metabolism, with the utilization of the animal's own fat and tissue protein. Woodyatt ('15-'16) in his experiments demonstrated such a marked increase in metabolic rate following dextrose injection.

From the sections of these tissues it was found that during the first 24 to 48 hours there is a marked decrease in the number of granules in the beta cells. From 4 to 5 days there is evidence of an increase in island tissue.

That this increase is due in part to growth in preexisting islands is evidenced by mitoses in beta cells in the larger islands, as observed in the tissue from the 4-day animal, no. 25, and from the 7-day animal, no. 26. The development of beta cells from centro-acinar cells and intercalated duct cells has also been observed in the tissue from the 5-day animal. Moreover, there is evidence in the tissue from the 4-day animal (no. 28) that even acinar cells may directly transform into island cells. Here are found acini among whose cells a single cell occurs which shows the properties of both acinar and beta cells. That is, a cell in the position of an acinar cell, containing a small amount of chromophile material at the base and zymogen granules toward the lumen, all characteristics of a differentiated acinar cell, at the same time is adjacent to a dilated capillary and contains specifically staining beta granules in the base of the cell toward the capillary. The mitochondria, instead of being the long filamentous type characteristic of the active acinar cell are shorter, approaching the small rod-like type, characteristic of the beta cell, but not fragmented or swollen as they are in the Mankowski cell.

Such unquestionable evidence of the direct transformation of acinar cells into beta cells has not been presented before. Many investigators have claimed to have observed such transitions. But when the criteria for these assertions are examined they are found to be inadequate.

Bensley ('15, p. 272), after reviewing the specific characteristics of the acinar and beta cells, pointed out:

Considering these properties of the cell it is obvious that to establish a transition between them we must find cells which partake of the properties of both, . . . . We cannot accept the presence of undifferentiated cells *per se* as evidence of transition but must insist on the intermediate phases which demonstrate progress and its direction.

Examining on the basis of these criteria the assertions of those authors who describe transition, we find that they fall into three classes: first, those who state that, as the acinous cell loses its zymogen and chromidial substances, it becomes more like an islet cell; second, those who have lightly and without due consideration interpreted the A cells as transitions because they frequently occur on the surface of the acinus; and third, those who perceived the logical necessities of the case and attempted to prove a real transition by showing the acquisition by the acinous cells of positive islet characters. The first class requires no discussion because the statements rest upon a negative interpretation of the islet cell. The second group has been sufficiently answered by the demonstration by Lane and others that the A cell was not an intermediate but a specialized cell with characters peculiar to itself. The third group, consisting of Mankowski and Laguesse, requires a more extended discussion.

Bensley goes on to show that the granular cells which Mankowski described as transitional cells and which are now called by his name are in reality degenerating forms of acinar cells whose mitochondria swell, become spherical and finally disappear in the cytoplasm, and whose zymogen granules disappear. He points out that the granules in these cells may be differentiated from the beta granules by their larger size, their intense staining and by their resistance to fixatives in which the beta granules are lost. The transition cells which Laguesse described in the normal pancreas Bensley was unable to confirm and suggested that these might be optical illusions of partially superimposed cells rather than actual independent intermediate cells.

Bensley considered that after ligation of the pancreatic duct in the guinea pig, the dedifferentiated acinous cells and cells of the ducts participated in equal measure in the regeneration of island tissue. He did not observe a direct transformation

of fully differentiated acinous cells into island cells, but he did not assert that this could not occur.

The demonstration, therefore, of positive characteristics, of position and cell constituents, of both acinar and beta cells within the same cell, by Bensley's cytological methods, would seem to fulfill his requirements for adequate evidence of the direct transformation of differentiated acinar cells into beta cells. This demonstration of a direct transformation of one specialized type of cell into another specialized type of cell is one more argument against the theory of irreversibility of differentiation.

Toward the end of the first week, compensatory and degenerative changes may occur within the same pancreas. At this period the beta cells generally contain many granules and these are larger than the normal granules of the beta cells. There are, however, a number of unusually large and a few small beta cells that contain very few granules. These beta cells, in the absence of a specific granule content, are difficult to distinguish from alpha cells which are also deficient in granules. Here the differentiation must be based largely on nuclear detail and this is not always reliable. There are a few cells, usually alpha cells, that show hydropic degeneration. All of the alpha cells stain paler than the normal tissue. In the acinar cells the mitochondria are swollen and some of the mitochondria contain large fat droplets.

Later, after 2, 3, or 4 weeks, the tissue appears more like the normal except that there may be many beta cells poor in granules, some containing unusually large granules, and the alpha cells are pale with an area staining dark with methyl green, close to the nucleus. The acinar tissue approaches the normal.

Like the experiments of Homans and Allen, these results indicate that increase in the blood sugar does affect the beta cells and may cause hydropic degeneration in them. But in contrast to their results are the compensatory increase in island tissue, the absence of complete hydropic degeneration of the beta cells and also the occurrence of degenerative changes in the alpha cells and acinar cells.



A possible explanation for these differences may lie in the fact that Homans and Allen removed the factor of safety by surgical reduction of the pancreas to a point where the excess of sugar constituted such a strain on the remaining beta cells that they degenerated and the sugar was thereafter not available for body metabolism but was largely excreted in the urine, due perhaps to insufficient insulin production. In the intact pancreas, on the other hand, the excess sugar is met with an increase in island tissue and perhaps insulin production, is rapidly oxidized so that the blood sugar level is rarely above that of a feeding animal and therefore constitutes but a moderate strain on the beta cells, but results in an increase in metabolic rate and upset in the fat and protein metabolism, which may be responsible for the later degenerative changes in the other types of cells. These changes, however, may be due to incomplete nutrition and vitamin deficiency.

It may be that larger amounts of sugar injected for short periods of time, or moderate amounts for longer periods would cause more extensive hydropic degeneration of the beta cells in the intact pancreas. This is at present a subject of further experimentation.

The staining methods used in this research are not adapted for the study of the delta cells discovered by Bloom and therefore no data have been accumulated as to their response to these experimental procedures.

#### SUMMARY

Continuous intravenous injection of moderate amounts of dextrose into guinea pigs for as long as 28 days indicate that:

1. There is a great amount of individual variation in the reaction of guinea pigs to continuous sugar in the blood.
2. It produces anorexia.
3. It produces no extensive hyperglycemia, glycosuria, or glycogen storage, but a marked loss in body weight, which is interpreted as an increase in basal metabolism.
4. At first the beta granules are exhausted in the islands.
5. Subsequently there may be an extensive increase in island tissue.

6. The number of beta cells may be increased: a) By mitoses of pre-existing beta cells; b) by transformation of duct and centro-acinar cells; c) by direct transformation of acinar cells.

7. Following the initial period of exhaustion followed by increase in island tissue there may be a period when degenerative changes occur.

8. The degenerative changes may involve both alpha and beta cells in the form of hydropic degeneration, and also the acinar cells in the form of swollen and fatty mitochondria.

9. In animals that survive the injection for longer than 2, 3 or 4 weeks both island and acinar cells approach the normal condition, although in the islands there may be many beta cells devoid of granules and alpha cells which stain poorly save for a dark staining cap near the nucleus.

10. No evidences of an established diabetes occur in animals injected with this amount of sugar even after 28 days.

#### CONCLUSION

When a moderate increase in the blood sugar level is maintained by continuous intravenous injection of dextrose, a diabetic condition does not result in the guinea pig. Although the beta cells in the islands of Langerhans appear to be affected by continuous injection of sugar, in many cases there is an intense compensatory increase in the island tissue. Beta cells may arise by mitoses from pre-existing beta cells, by transformation of duct and centro-acinar cells and even by direct transformation of acinar cells. With this increase in island tissue and insulin secretion, there appears to be an increase in metabolic rate resulting in considerable loss of body weight. Hydropic degenerative changes do occur in the alpha cells and duct cells as well as in the beta cells.

I wish to acknowledge my indebtedness to Dr. Sylvia H. Bensley, Dr. N. L. Hoerr and Dr. R. R. Bensley for their generous advice and encouragement during the course of this work, and to Mr. R. D. Bensley for the photomicrographs.

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## PLATE 1

### EXPLANATION OF FIGURES

The optical system used for figures 4 to 10 consisted of a Zeiss apochromatic oil immersion lens H.I.  $\times 35$ , 0.85 aperture and a Leitz  $\times 8$  ocular with a bellows length which gave an initial magnification of  $\times 400$  (except where otherwise indicated). A subsequent enlargement of two diameters was made by enlargement of the negative.

4 and 4 a Four micra section of normal guinea pig pancreas, fixed in formalin-Zenker, stained in neutral gentian. Cells in an island of Langerhans.  $\times 800$ . *a*,  $\alpha$  cells. *b*,  $\beta$  cells with many granules. *bn*, nucleus of  $\beta$  cell containing few granules.

5 and 5 a Four micra section of pancreas of guinea pig no. 4 injected for 1 day with dextrose, fixed in formalin-Zenker, stained in neutral gentian. Island cells.  $\times 800$ . *b*,  $\beta$  cell with vacuoles. *bn*, nuclei of closely packed small  $\beta$  cells devoid of granules.

6 and 6 a Four micra section of pancreas of guinea pig no. 28 injected for 4 days with dextrose, fixed in formalin-Zenker, stained in neutral gentian. Acinar cells.  $\times 950$ . *ac*, acinar cell containing zymogen granules near the lumen and small  $\beta$  granules at the base of the cell.

7 and 7 a Four micra section of pancreas of guinea pig no. 11 injected for 5 days with dextrose, fixed in formalin-Zenker, stained in neutral gentian. A peripheral island in relation to acini.  $\times 800$ . *ac*, acinar cell. *acs*, group of acinar cells. *b*,  $\beta$  cell rich in granules; *b'*,  $\beta$  cell containing small granules.





## PLATE 2

### EXPLANATION OF FIGURES

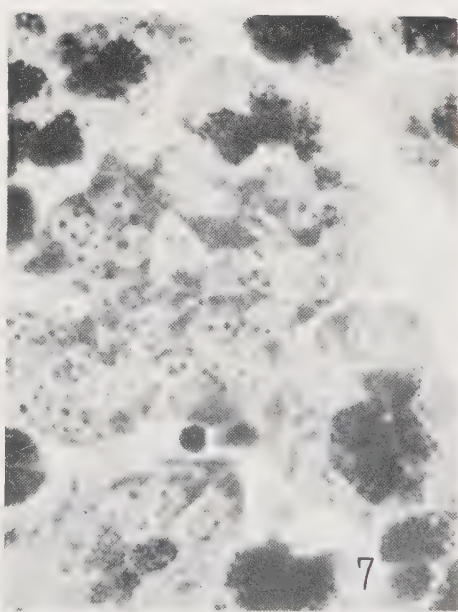
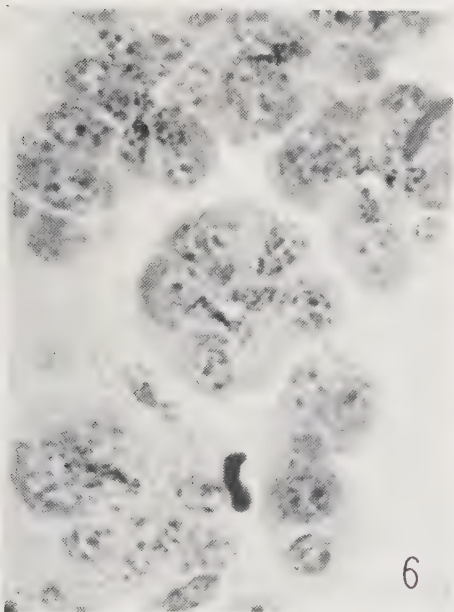
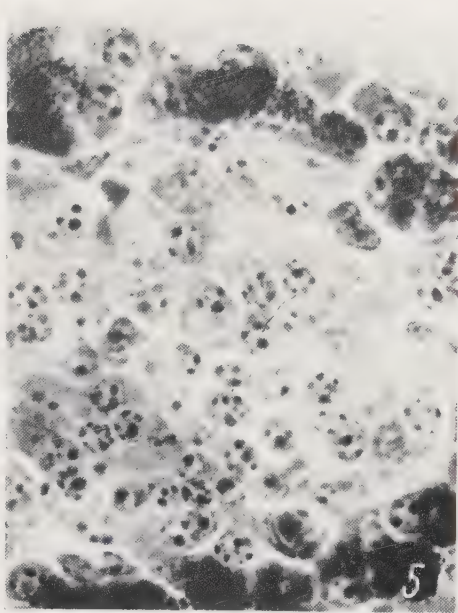
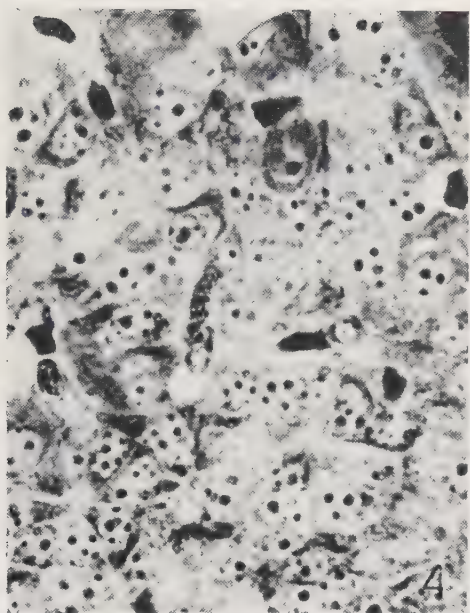
8 and 8 a Four micra section of pancreas of guinea pig no. 17 injected for 7 days with dextrose, fixed in formalin-Zenker, stained in neutral gentian. The edge of an island.  $\times 800$ . *ab*,  $\beta$  cell lacking granules. *gb*,  $\beta$  cell rich in large discrete granules. *hd*, cell showing hydropic degeneration.

9 and 9 a Four micra section of pancreas of guinea pig no. 17, fixed in Bensley's AOB, stained in aniline acid fuchsin and methyl green. The edge of an island.  $\times 800$ . *a*, pale staining  $\alpha$  cell. *bn*, nucleus of  $\beta$  cell. *sm*, swollen mitochondria in acinar cells.

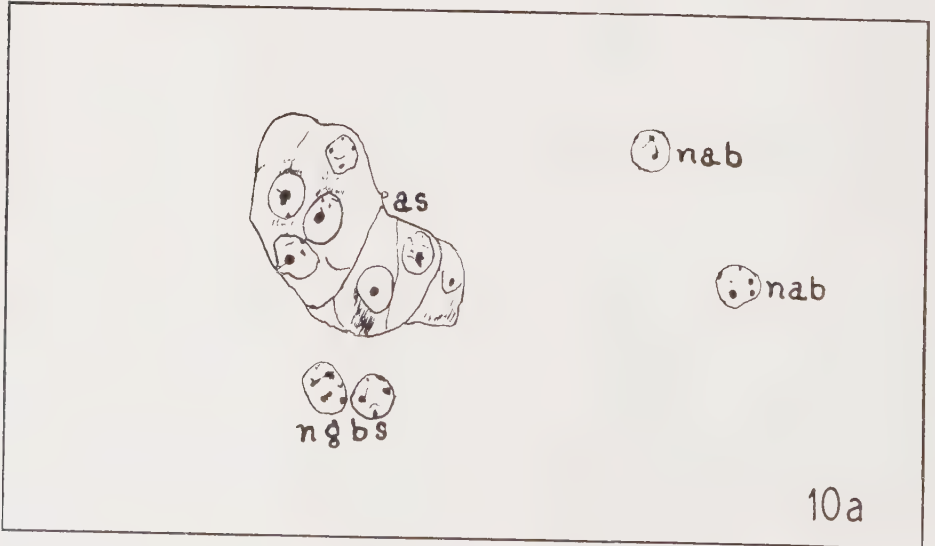
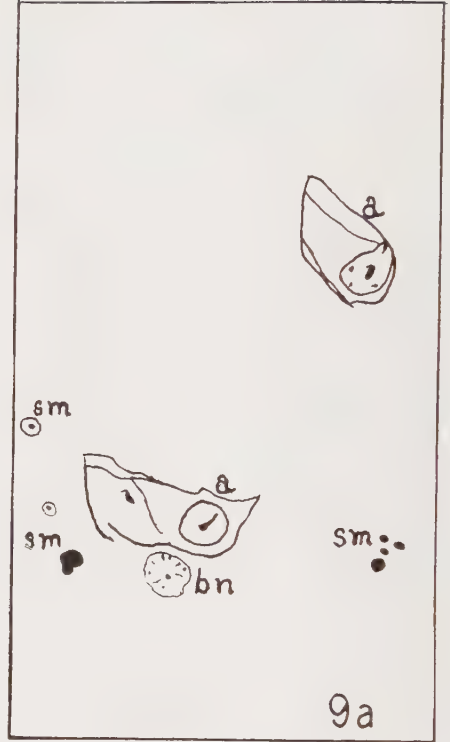
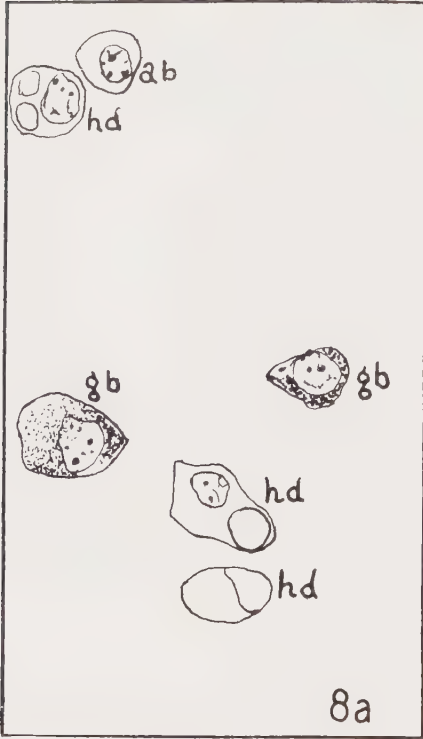
10 and 10 a Four micra section of pancreas of guinea pig no. 20 injected for 28 days, fixed in formalin-Zenker, stained in aniline acid fuchsin and methyl green. Island cells.  $\times 800$ . *as*, group of pale-staining  $\alpha$  cells with dark-staining area near the nucleus. (Compare these with figs. 6 and 8 a.) *nab*, nucleus of  $\beta$  cell poor in granules. *nab.s*, nuclei of  $\beta$  cells rich in large discrete granules.

CONTINUOUS INJECTION OF DEXTROSE  
C. ARTHUR WOERNER

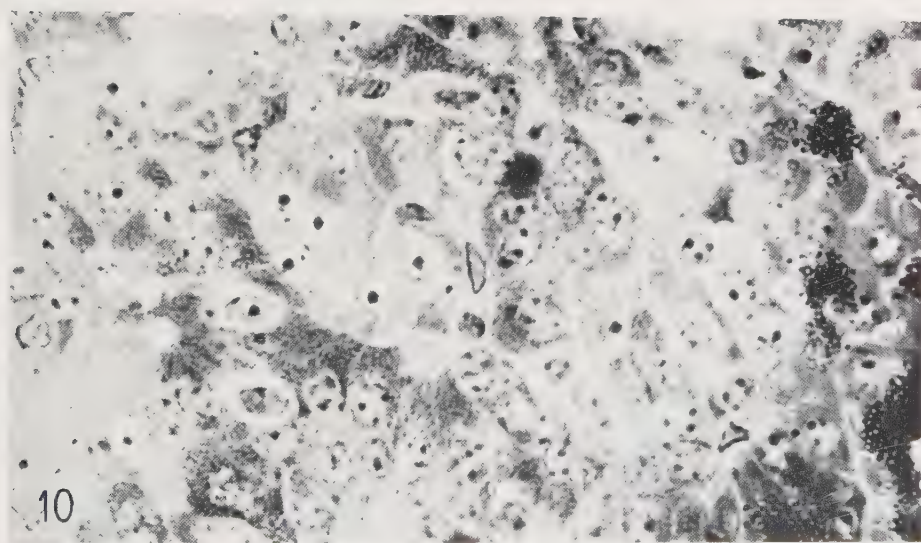
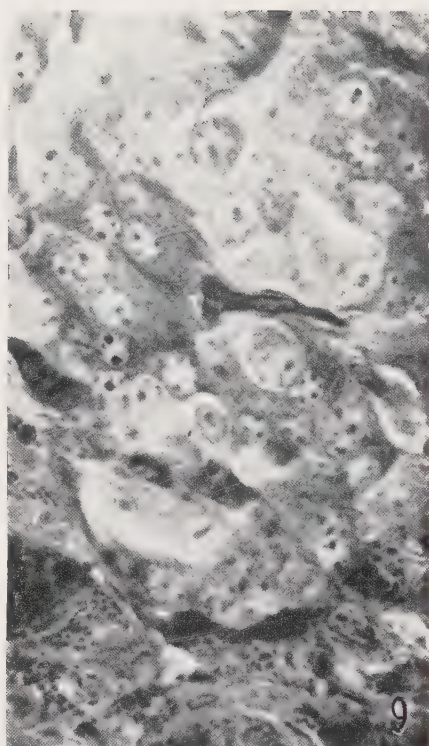
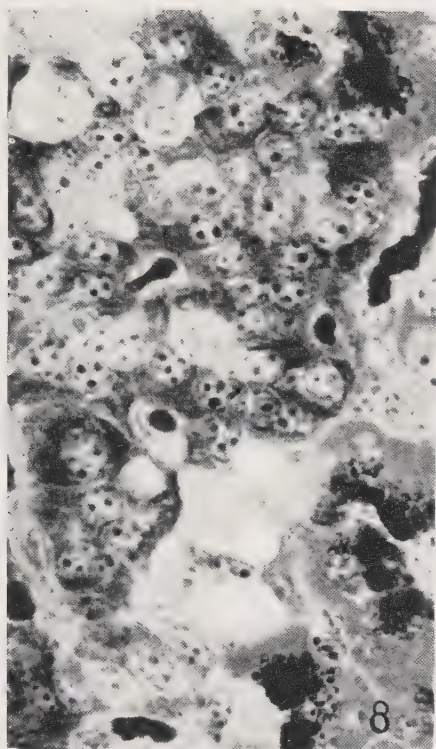




CONTINUOUS INJECTION OF DEXTROSE  
C. ARTHUR WOERNER







### PLATE 3

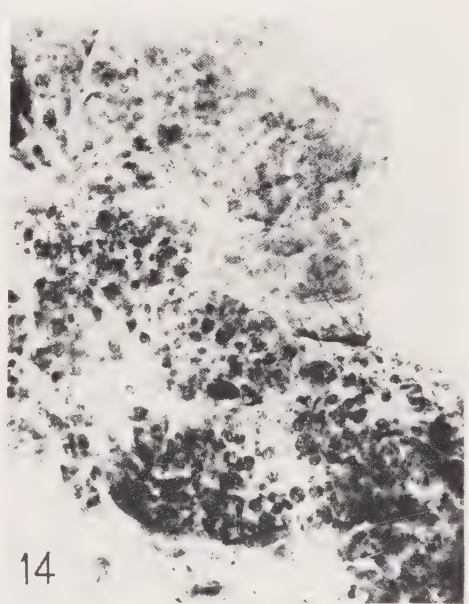
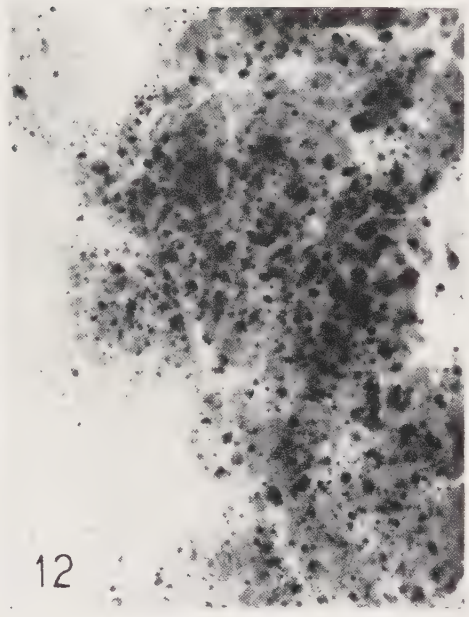
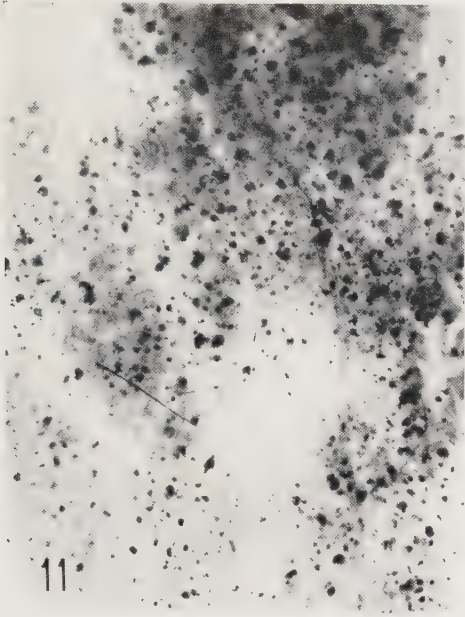
#### EXPLANATION OF FIGURES

The optical system used for figures 11 to 14 consisted of a Zeiss 32 mm. planar lens with bellows length to give a magnification of  $\times 10.75$ .

11 and 12 From a whole mount of the pancreas of a normal animal perfused with Janus green.  $\times 10.15$ .

13 From a whole mount of the pancreas of guinea pig no. 26, injected for 7 days with dextrose and perfused with Janus green.  $\times 10.75$ . Note the increase in the large islands and the increase in the acinar type of island.

14 From a whole mount of the residual pancreas of guinea pig no. 27, injected for 7 days with dextrose after partial pancreatectomy, and perfused with Janus green.  $\times 10.75$ . Note the acinar type of islands.







# SOME EFFECTS OF TESTOSTERONE AND TESTOSTERONE-PROPIONATE IN THE RAT <sup>1</sup>

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## ONE FIGURE

The availability of androgens as pure chemical compounds makes it possible not only to compare their effects in the organism with the effects of hormones secreted by the testes but also to extend our knowledge of their action. It is important, therefore, to study closely the comparative responses of the organism under the influence of naturally secreted hormone and the pure chemical substances, and our earlier studies on androsterone (Moore and Price, '37) are continued here with the utilization of testosterone and testosterone-propionate. Appropriate procedures provide at least a rough means of determining changes in the secretory activity of the normal testes.

The present brief report of experiences with these two pure chemical substances is based upon a careful study of more than 200 albino male rats born in our breeding colony. Since inevitable variation is believed to be less among litter mates than among animals chosen at random our judgment of effects has been based upon differences exhibited within the litter by treated as compared with untreated animals.

It is a genuine pleasure to acknowledge the courtesies extended during this study by Dr. Gregory Stragnell of Schering Corporation in supplying the pure synthetically prepared androgenic compounds.

<sup>1</sup> This investigation has been aided by a grant from the Rockefeller Foundation to The University of Chicago.

## A. EFFECTS IN CASTRATE MALES

The determination of effects of testosterone and the propionate have been based on fresh weights of individual parts as compared with those recovered from normal litter mate controls, and upon the histological character of the tissues, especially the ventral lobe of the prostate gland and the seminal vesicles. With the aid of a binocular dissecting microscope all tissues have been removed immediately upon sacrifice of the animal and weighed to fractions of a milligram on a torsion balance, after careful removal of all fat and connective tissue.

In young males sacrificed on day 15 or earlier, daily injection periods resulted in a precocious development of the accessory organs as judged by weight increases, and also by a precocious secretory differentiation; seminal vesicles, which normally show weak secretion granules only about day 35 or later, differentiate into a secretory type of seminal vesicle by day 18 that is indistinguishable microscopically from that of mature adult males. Daily injections of 0.4 mg. testosterone into males after castration on day 15 and sacrifice on day 35, result in seminal vesicles weighing 1900% more than those of untreated litter mate controls. Testosterone propionate, as will be shown below, is even more effective.

The microscopical character of the normal and castrate prostate and seminal vesicle of the rat has been described earlier (Moore, Price and Gallagher, '30; Moore, Hughes and Gallagher, '30). It may be mentioned in general that both testosterone and the propionate in effective doses either maintain the normal secretory state of these organs in castrated males of any age, or restore long-time castrate tissues to the normal secretory state. No qualitative differences have appeared in these tissues conditioned by chemical androgens that would distinguish them from tissues of normal males when proper dosages are employed.

On adult males, dosages of testosterone and the propionate required for testicular replacement have been determined from daily injections begun immediately after castration and

continued for a period of 20 days. The two criteria employed to determine testicular replacement, and representing different dosage requirements, have been 1) the quantity of substance administered daily that is necessary to maintain the histological normality of the ventral prostate and seminal vesicles; 2) the daily dosage requirement for maintenance of the normal fresh weight of the seminal vesicles. Requirements for the second indicator are usually about three to four times requirements for maintenance of histological normality.

### *Histological normality*

Histological normality of the ventral prostate consists in the maintenance or production of an active secretory epithelium in acini, the cells of which contain well-defined light areas marking the position of the Golgi apparatus midway between the nucleus and luminal end of the cell. Such diagnostic light areas make their appearance in ventral lobe cells about day 15 (Price, '36); they are prominent from day 30 throughout the hormone secreting period, and in adults they disappear about the fourth day after castration. They are clearly revealed in tissues after Bouin fixation and Harris hematoxylin stain. Histological normality of seminal vesicles consists of the presence of a lining epithelium of high columnar cells containing definite secretion granules, surrounded by clear 'halo' areas in stronger preparations, easily demonstrated by Bouin fixation and Ehrlich's hematoxylin stain. These granules make their first appearance in normal animals about days 35 to 40 depending upon the state of hormone secretion, or degree of maturity, and in castrates they disappear approximately 48 hours after castration.

More than fifty animals were utilized in the determination of dosage requirements of testosterone and the propionate to maintain a normal histology of the ventral prostate and seminal vesicles in castrated adults, as well as requirements to maintain normal weight of seminal vesicles. Ventral prostates required from 30 to 50 gamma daily of testosterone for

histological normality and the lowest effective dose of propionate was not determined. Seminal vesicles contained well-defined secretion granules with a daily dose of 200 gamma testosterone and with somewhat less than 50 gamma of propionate. Maintenance of seminal vesicle weight in adults was obtained with 600 to 700 gamma daily and with 150 gamma of propionate.

*Growth maintenance of seminal vesicles.* It early became evident that dosages of chemical androgens required to maintain the growth of seminal vesicles equal to that of normal litter mates differed with age of the animals. The observations on one litter consisting of seven males, the castrates of which were treated with testosterone between days 30 and 50

TABLE 1

*Effects of testosterone in castrated rats. Castration day 30; injections daily days 30 to 49; sacrifice day 50*

|               | Weight, grams                |                     | Per cent change<br>sem. ves.<br>from normal | Prostate-sem.<br>ves. ratio |
|---------------|------------------------------|---------------------|---------------------------------------------|-----------------------------|
|               | Total<br>prostate<br>complex | Seminal<br>vesicles |                                             |                             |
| Cast. 0.1 mg. | 0.1354                       | 0.0188              | — 47                                        | 7                           |
| Cast. 0.2 mg. | 0.3327                       | 0.1606              | + 352                                       | 2                           |
| Cast. 0.4 mg. | 0.4416                       | 0.4504              | +1168                                       | 0.9                         |
| Cast. control | 0.0384                       | 0.0054              |                                             | 7                           |
| Cast. control | 0.0501                       | 0.0066              |                                             | 7                           |
| Norm. control | 0.1166                       | 0.0257              |                                             | 4.5                         |
| Norm. control | 0.1971                       | 0.0454              |                                             | 4.5                         |

are presented in table 1 as an example of treatments given other litters of different ages. Ten litters including more than fifty males have been examined directly for these determinations and many animals of litters used for other purposes afforded additional data. For each litter the period of treatment was 20 days with injections of castrates beginning immediately after operation, the litters being sacrificed at ages 35 to 90 days; the rat is essentially mature by the eightieth or nintyeth day of life, since it readily sires young and has attained a mature prostate-seminal vesicle ratio.

Attention to table 1 reveals the average seminal vesicle weight of two normal males on day 50 to be 35 mg. in comparison with 6 mg. in untreated castrates. Seminal vesicles



of the castrate receiving 0.2 mg. testosterone daily showed a weight increase 352% greater than the normal litter mate average, but in the one receiving 0.1 mg. daily the seminal vesicle weight was 47% below that of the normal controls. Other litters revealed that the growth-replacement dose of testosterone between the thirtieth and fiftieth day was approximately 0.15 mg. daily. In table 2 a summary of observations is given for replacement dosages for both testosterone and t-propionate for different 20-day periods during the age of most rapid growth of reproductive parts. It can be seen that whereas 0.1 mg. daily dose of testosterone serves as an adequate substitution for the testicle in promoting growth of seminal vesicles from day 15 to day 35 it requires a daily dose

TABLE 2  
*Daily dosages for growth maintenance of seminal vesicles, during various 20-day periods*

| <i>Autopsy age, days</i> | <i>Testosterone</i> | <i>T-propionate</i> |
|--------------------------|---------------------|---------------------|
| 35                       | —0.1 mg.            | ....                |
| 50                       | 0.15 mg.            | 0.05 mg.            |
| 60                       | ....                | 0.08 mg.            |
| 70                       | +0.30 mg.           | 0.08 mg.            |
| 80                       | +0.60 mg.           | 0.15 mg.            |
| 90                       | ....                | 0.15 mg.            |

of slightly more than 0.6 mg. between days 60 and 80 to maintain growth of these organs in castrates equivalent to litter-mate controls. A similar trend is shown for t-propionate but dosage requirements are much below those of testosterone. The greater effectiveness of the former has been pointed out by most workers using these substances.

*Prostate-seminal vesicle ratio.* The question is pertinent whether testosterone or the propionate has a selective effect upon one end organ or provides a general stimulation equivalent to that of testis-secreted hormone. This question was examined by Moore and Price ('37) with respect to the effects of androsterone, basing judgment upon an analysis of the relative weights of the total prostate complex to the seminal vesicles. It was determined on normal males of different ages

that the prostate-seminal vesicle ratio value prior to the onset of intensive hormone secretion was high and as testes secreted more hormone there was a gradual decline from a value of 10, or greater, to a value of 1 at an age of approximately 80 to 90 days. Subsequent to this period and throughout reproductive life this value constituted the normal one, and whereas the ratio value declined with approach to maturity it was surprisingly constant for the litter. In table 1 the determination of the prostate-seminal vesicle ratio is included for one litter. In this litter sacrificed on day 50, each of two normal males showed a ratio value of 4.5 whereas the value for two castrates was 7. The castrate injected with doses of testosterone inadequate to maintain normal growth of seminal vesicles (0.1 mg.) revealed a ratio value greater than that for normal litter mates but a second castrate receiving an excessive dosage (0.4 mg.) exhibited a ratio value much below the normal for the age and entirely equivalent to that of mature adult males (0.9). The dosage of 0.2 mg. was more than sufficient for testis substitution (seminal vesicle weight 352% above control) and gave a ratio value approximating that of maturity. Whereas in this particular litter an exact growth-substitution dosage was not injected, other litters have shown that similar to the effects of androsterone, testosterone and the propionate in substitutional doses produced a prostate-seminal vesicle ratio value equivalent to normal litter mates. This again emphasizes the effect of the chemical androgens as equivalent to that of the naturally secreted hormone insofar as these methods are capable of demonstrating. With adequate replacing dosages they fail to exhibit a more selective stimulation on one end organ than does normally secreted testis hormone.

*Prolonged effects.* The question whether one compound exerts a prolonged effect on the mammalian end organs was briefly investigated by a study of the microscopic condition of tissues from forty adult males. Precautions were observed in injecting dosages of androsterone, testosterone and t-propionate into castrates for periods of 20 days that would

maintain the normal seminal vesicle weight and not represent many times the actual requirements for this purpose. Whereas weights of tissues were carefully recorded, chief dependence was placed upon the histological state of the prostate and seminal vesicles.

In ordinary untreated castrates, in which the hormone source is completely eliminated at once, marked castration changes appear within 4 days in the prostate and earlier in seminal vesicles. Injection of chemical androgens in an oil solution naturally provides residual unabsorbed active material for some time after the last injection.

Castration changes similar to the 4-day untreated castrates appeared usually by the sixth to seventh day after the last injection of androsterone and testosterone but injections of testosterone propionate produced effects observable for slightly longer periods after discontinuance of injection.

Thus in one group of twelve adult males of similar age and size, but not litter mates, ten were castrated and injected immediately with 0.2 mg. t-propionate daily for 20 days. Two injected castrates and two untreated normals were sacrificed 1 day after the last injection when it was determined that seminal vesicles of injected castrates were 70% heavier than those of the untreated males (averages of two each); thus the dosage for all injected animals was slightly in excess of a replacing dose. Histologically the prostates and seminal vesicles of the normals and injected castrates were indistinguishable. Two injected castrates were sacrificed thereafter on days 5, 10, 13 and 17 after the last injection. The prostates and seminal vesicles of animals killed 5 days after the last injection were histologically normal but those sacrificed on day 10 showed decided castration changes; those killed 13 and 17 days after the last injection revealed castration changes approximating those found in ordinary castrated males on about day 10 following operation.

In a second group of eight males (litter mates) six were castrated on day 70 and injected daily until day 89 with 0.15 mg. t-propionate, with sacrifice of two untreated normal

and two injected castrates on day 90 (1 day after last injection). This dosage proved to be almost an exact replacement dose since average weight of injected castrate seminal vesicles was only 19% heavier than those from untreated normal males. Histologically, prostates and seminal vesicles from the normal and injected males were indistinguishable. Two injected castrates were sacrificed on days 8 and 11 after the last injection. By the eighth day definite castration changes were evident and by the eleventh day a grade of involution was present that would correspond to approximately the eighth day of castration in ordinary untreated males.

Thus testosterone-propionate administered in approximate replacement doses, and not in large overdoses, showed but a slight prolongation of effect. Ordinary untreated castrate males attain a degree of castration change on the sixth to eighth day in the prostate and seminal vesicles that makes its appearance on approximately 8 to 11 days after the last of a series of injections. This slightly prolonged effect of not more than 5 to 6 days at most is probably due in part at least to slow resorption from oil residues in subcutaneous pockets hence it appears fair to conclude that prolonged effects in the castrate rat are not evident for more than approximately 3 days.

*Initiation of copulation.* Testosterone-propionate will not only maintain or repair accessory reproductive organs in castrates but its administration results also in the awakening of desire and ability to copulate when castration has been performed before germ cell production.

Three males of a group of five were castrated on the thirtieth day of life and caged with two normal controls for a period of 6 months. Two of the three received 22 daily injections of t-propionate, one 0.5 mg., the second 1.0 mg. daily; the third castrate was maintained as an untreated control. Within 2 weeks from the first injection the two treated males exhibited a normal response to females in heat and executed repeated and vigorous copulations with ejaculation; the untreated castrate showed no interest in females.



When sacrificed the day following the twenty-second injection average seminal vesicle weight of the two treated castrates was found to be 828 mg. as compared with 5 mg. for the un-injected castrate (increase of 16,000%) and 740 mg. for normal controls (increase of 11%). Daily treatments with 0.5 mg. apparently induced a maximal growth response since 1.0 mg. doses gave a similar seminal vesicle weight.

In this group, therefore, males castrated prior to the onset of hormonal activity and remaining as castrates from 30 days to an age of 7 months, were induced to develop into a state such that the copulation pattern was entirely characteristic of normal males and the accessory organs had developed slightly above the normal controls as a result of the 3 weeks of treatment.

TABLE 3

*Effects of t-propionate in normal male rats. Injections, days 20 to 39; sacrifice, day 40*

| <i>Daily dose</i> | <i>Testis weight, grams</i> | <i>Per cent decrease from control</i> | <i>Vent. prost., grams</i> | <i>Per cent increase over control</i> | <i>Sem. ves. weight, grams</i> | <i>Per cent increase over control</i> |
|-------------------|-----------------------------|---------------------------------------|----------------------------|---------------------------------------|--------------------------------|---------------------------------------|
| 0.05 mg.          | 0.3538                      | —72                                   | 0.0664                     | 61                                    | 0.0910                         | 712                                   |
| 0.10 mg.          | 0.3140                      | —75                                   | 0.1026                     | 150                                   | 0.3126                         | 2691                                  |
| 0.50 mg.          | 0.7474                      | —40                                   | 0.1824                     | 300                                   | 0.7090                         | 6230                                  |
| 1.00 mg.          | 1.0228                      | —19                                   | 0.1848                     | 350                                   | 0.8156                         | 7182                                  |
| Control           | 1.2110                      |                                       | 0.0432                     |                                       | 0.0106                         |                                       |
| Control           | 1.3146                      |                                       | 0.0386                     |                                       | 0.0118                         |                                       |

#### B. EFFECTS ON THE TESTICLE

Data were obtained on the effects of these chemical androgens on the testicle from eighteen litters of animals consisting of more than eighty-five males, the majority of which received treatments with testosterone propionate. In each litter the injected members were compared with their normal brothers and the 20-day periods of treatment were so arranged as to terminate within the first 80 days of life which covers the prepuberal period.

In table 3 will be found some details from one litter sacrificed on the fortieth day of life subsequent to a 20-day period of injection with four dosage levels of testosterone-propionate.

It can be noted that testis weights for each of the four treated males were appreciably below either of two untreated litter mates. Compared with the average normal testis weight that of the male receiving 0.05 mg. daily showed a reduction of 72%, or weighed 28% of normal, whereas treatments with 1.0 mg. daily reduced testis weight by but 19%. This production of less and less damage as the dosage is increased has been quite characteristic especially for the propionate. The

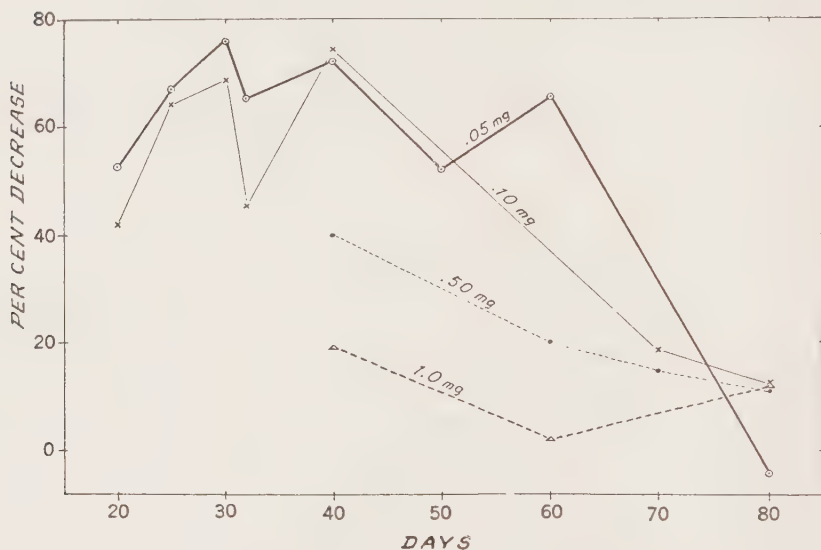


Fig. 1 Effect of testosterone propionate in suppression of testis growth. Ordinate represents percentage decrease in fresh weight of testis; abscissa represents age in days at autopsy, preceded by a 20-day period of injection. Each point shows percentage decrease in testis weight in treated males as compared with normal untreated litter mates. Amount of daily administration indicated on curves.

effects on the accessory organs have been usually one of increased effect with increased dosage. In the litter under consideration ventral prostate weight increases above normal controls were from 61% to 350% and seminal vesicle increases were from 712% to 7182%.

The effects on the testicle of four dosage levels of t-propionate for different 20-day periods have been summarized in a graph (fig. 1) each point on which represents percentage

change in testis weight determined from comparisons with normal litter mates.

Considering the general aspects of this graph, and that of numerous cases with a dosage range from 0.05 mg. to 1.0 mg. and not represented on the graph, it becomes evident 1) that testosterone-propionate in this dosage range induces marked loss in testis weight. This is evident in twenty-one of the twenty-two treated males represented. One litter exception occurred in which the injection period was 11 instead of 20 days (8 to 19); in this litter testes from three treated males were slightly heavier than those from the two control males. 2) In general the injurious effects appear in the reverse order rather than parallel with dosage since usually 0.05 mg. t-propionate induced more severe injury than 1.0 mg. daily doses. With ordinary testosterone, injuries are induced with similar dosages but the reverse order of effects with dosage is less apparent. 3) The harmful influences upon the testis appear to diminish as the animal approaches maturity. This is evident not only from the graph of effects after propionate treatment but it is likewise evident after ordinary testosterone. Thus testosterone in daily dosages of 0.1 mg. 0.5 mg. and 1.0 mg. and sacrifice on day 50 caused a decrease in testis weight of from 58% to 67% in contrast to sacrifice on day 70 in which decreases of but 9% and 13% were recorded.

Two males receiving 2.0 mg. t-propionate daily from days 70 to 90 registered decreases of but 1% and 10%.

The effect upon the testicle indicated by decrease in weight involves real tissue damage in the more severe cases. In testes just approaching the period of spermatozoon differentiation tubules are much reduced in size and show considerable sloughing of cells into the lumen. Treated males having attained the age of spermatozoon differentiation may show pronounced delay in development as well as considerable actual injury. The treatment does not prevent the testes from ultimately differentiating spermatozoa since considerable weight reduction may be accompanied by a few tubules with spermatozoa though the percentage of tubules representing such development is markedly less than the controls.

In order to determine whether testosterone or t-propionate exhibited any tendency to stimulate spermatogenesis a number of litters were so injected as to provide sacrifice on approximately day 32 since by day 36 untreated males possess tubules showing the 'sperm-head' stage (Moore, '36). No evidence has been forthcoming that either of these chemical androgens stimulated spermatogenetic activity and detectable influences in every case have been of an injurious rather than a stimulating nature.

#### DISCUSSION

The comparative effects of the chemical androgens, testosterone and testosterone-propionate, and normally secreted testis hormone are remarkably similar when prostate gland and seminal vesicles of rats are used as the test objects. These substances are capable of producing any grade of effect from barely detectable responses of the end organs, to conditions indistinguishable from normal litter mates, or to marked precocious growth and secretory responses when appropriate daily dosages are employed. Whereas qualitative differences in effects of these two chemical substances have failed to be noted in this study the quantitative differences noted by numerous investigators have been evident. Testosterone-propionate per unit weight has shown an activity approximately four times greater than testosterone.

Adequate growth-substitutional dosages of these two substances have been found to promote types of relationships between the weight of the prostate and seminal vesicle that exist in normal litter-mate controls of young or adult ages. These findings are in agreement with those obtained from treatments of castrate rats with androsterone and it is to be emphasized that though dosages below requirements for testis substitution on growth maintenance of seminal vesicles will appear to selectively stimulate the prostate because of its lower threshold of response, adequate substitutional dosages provide a prostate-seminal vesicle ratio equivalent for the age. Reports that the chemical androgens exert a selective stimulation on one end organ rather than a general stimulation



characteristic of testis-secreted hormone are believed to be based upon the use of dosage levels too low to adequately stimulate seminal vesicles, while being sufficiently high to permit the lower threshold reacting prostate to develop normally (Callow and Deanesly, '35; Korenchevsky et al., '35; Deanesly and Parkes, '36; Parkes, '36; Miescher, Wettstein and Tschopp, '36; Tschopp, '36). The shifting of the ratio value by different dosages of testosterone is illustrated in table 1 wherein for the terminal age of 50 days 0.1 mg. would selectively stimulate the prostate and give an aberrant ratio value higher than normal, 0.2 and 0.4 mg. representing more than substitutional dosages, reduce the value lower than normal and shift relative weights to those of normal adults rather than those characteristic for the real age of the litter.

The adequacy with which these chemical androgens substitute for the testis and the quantitative variation in growth-replacing doses for varying ages, suggests that such procedures provide a rough measure of secretory intensity of normal testes. It has been pointed out (Price, '36; Moore and Price, '37) that seminal vesicles of normal male rats grow slowly for approximately the first 35 or 40 days of life when the curve of weights ascends sharply until approximately 80 to 90 days of age when the ascent is more gradual. Data on replacement dosages herein presented indicate that secretory activity of testes gradually increases during prepuberal development up to the level maintained in adults and that such a stage is reached at about 80 days of age. The same line of reasoning suggests that the pituitary gland, the normal incitor of testis activity, likewise begins to release more and more gonad activating hormones. Clark ('35) demonstrated that the pituitary of male rats gradually increased in gonadotropic potency from birth to puberty. As shown by treatments with gonadotropic hormone injections the amount released is by no means sufficient to induce the full secretory capacity of the testes.

These considerations suggest that puberal development is a process characterized by a gradual incremental increase in

testis hormone secretion and that as the high level of secretion is attained at approximately 80 days it fails to increase further, normally, but is capable of being increased by proper treatments. With the establishment of this normal peak level of activity, regulatory forces within the organism appear to become operative and probably consist at least in part of a check on pituitary activity by high testis hormone levels. Hooker ('37) extracted testicles of different aged bulls and considers that hormone secretion is a gradually increasing function from 1½ months to 5 years of age, after which the function gradually declines. In the rat the high point is attained within 3 months and appears to remain fairly constant during reproductive life.

It has been indicated that testosterone-propionate exhibits effects of longer duration than other androgens (Miescher, Wettstein and Tschopp, '36; Parkes, '36) and it has been of interest to compare its effects not only with those from testosterone and androsterone but also with the normally secreted hormone.

Castration of normal adult male rats is followed by such rapid reduction of the hormone level as to induce definite castration changes within a period of 4 days on the prostate gland and even earlier on seminal vesicles. In this case the source of hormone is removed suddenly and completely and no storage in the organism is apparent. Injections of chemical androgens in an oil medium provide a residual source of hormone for approximately 2 days since a grade of castration change appears after cessation of injections of testosterone and androsterone about days 6 to 7 that appears after ordinary castration on days 4 to 5.

In experiments here reported care has been exercised to employ growth-substitutional doses in order that the end organ development would be equivalent to that in the normal animal. Activity of secreting cells in the end organs has served as the principal criterion of effect instead of gross weight of organs. By these methods castration changes following the last of a series of injections with the propionate

appear to have been delayed for approximately 3 to 4 days longer than after treatments with other chemical androgens or after ordinary castration. This short prolongation of effectiveness is presumed to represent slower absorption of t-propionate from the oil.

The rapidity with which full-sized seminal vesicles can be produced in prepuberally castrated males untreated for 6 months is remarkable. Within 3 weeks from beginning treatment with propionate active secretion has been initiated as well as an actual increase in weight of 160 times as compared with untreated castrates; the vesicles attained a size and weight in 3 weeks that had been attained in the normal control at the age of 7 months.

Copulatory ability was developed to its full capacity within the 3-week period of injections. This is real copulatory awakening since normal males on the average experience first copulation only about 60 days of age (Stone, '24, '27) and castration had been performed on day 30. Despite the fact Stone ('27) determined copulatory ability was evidenced 8 months after castration in one rat, his animals were castrated about day 90 and after they had experienced copulation. Even so, 74% of a group of forty-five males castrated at the age of 90 days had lost copulatory ability by the end of the fourth month after castration. The absolute perfection of the copulatory act on the part of males treated with testosterone-propionate, and the fact ejaculations occurred, leave no doubt of the entirely normal character of the reaction developed at the end of a castration period of more than 6 months and developed for the first time. De Fremery and Tausk ('37) found that prepuberally castrated rabbits developed copulatory ability 7 months after castration after injections of 10 mg. of t-propionate daily for a period of 11 to 13 days. Unpublished observations (Moore) show that guinea pigs castrated at 30 days of age and injected 6 months later developed copulatory ability (with ejaculations) after daily injections of 0.3 mg. and 0.5 mg. testosterone-propionate.

The injurious effect of testosterone and t-propionate upon the testes of males during the rapidly growing period and their stimulating effect on the accessory organs coincides with earlier findings on the effect of bull testis extracts (Moore and Price, '32) and the effects of androsterone (Moore and Price, '37). Testes from treated males were materially reduced in weight; differentiation was arrested; destruction and sloughing of cells of the germinal line into the seminiferous tubules was prominent; tubule diameter was reduced and spermatozoon formation completely inhibited or greatly delayed. Two points of similarity of effects with those after androsterone treatments were 1) that the harmful influences from given dosages become less evidence as the animal approaches maturity and 2) relatively large dosages fail to materially affect the testes of mature adults. Differing at least in degree from other androgens the propionate exhibited the somewhat perplexing property of being more injurious in lower dosages. The figure shows that for most periods during prepuberal life daily doses of 0.05 mg. induce testis decline and injury to a greater extent than those of 1.0 mg.

Interpretations of these phenomena are not clear at the present time. Our earlier suggestions (Moore and Price, '32) were to the effect that deleterious action of androgens upon the testicle rather than being a direct effect was indirect by virtue of a suppressive action upon the pituitary, or one which led to an insufficient amount of pituitary hormone being available to stimulate normal testis function. Pituitary glands from normal rats treated with androsterone, which resulted in testis damage, were found to have diminished gonadotropic activity in infantile female mice (Moore and Price, '37). Hertz and Meyer ('37) have shown also that chemical androgens injected into the castrate partner of a parabiotic pair effectively prevented the usual stimulation of the normal female of the pair, presumably by virtue of preventing excessive pituitary activity that follows in the castrate partner and expressed in the normal parabiont partner. These and numerous other data, considerable of which are cited in



former papers, suggest the effect as one of pituitary modification, and the declining effects with ageing can be understood as perhaps due to a less easily modified pituitary gland. The general hypothesis contains no element of explanation, however, for the decline in effectiveness with elevation of dosage as has been found after treatments with the propionate.

The suggestion of absence of direct effect of the androgenic substances upon the testis might appear to deserve modification when consideration is given to the fact that 1) androgenic substances prevent testis involution when injected immediately after hypophysectomy (Walsh, Cuyler and McCullagh, '34; Nelson and Gallagher, '36; Wells and Gomez, '37) and 2) that treatment with androgenic substances results in heightened testis activity in the seasonal breeding mammal (*Citellus*), leading to unusually early production of spermatozoa (Wells and Moore, '36).

With regard to treatments of hypophysectomized males with various androgens it should be mentioned first that, in agreement with the early unpublished findings of Vatna (Moore and Price, '32) using bull testis extract, repair of hypophysectomy damage to the testis was not effected. A consideration 1) of the capacity of androgens to prevent involution after hypophysectomy but inability to correct damage to the testis after its development, and 2) the fact that progestin (Nelson, '36) or yeast extract (Hisaw, Greep and Fevold, '36) likewise prevent testis damage when given immediately after hypophysectomy raises the question whether androgens have a direct effect upon the testicle.

Reproductive conditions in seasonally active forms admittedly differ from those in continuous breeders such as the rat. It was determined that administration of androgens to the normal but sexually inactive ground squirrel was followed by heightened testicular activity and germ cell production (Wells and Moore, '36) even in case the animal had been subjected to hypophysectomy (Wells and Gomez, '37). Ordinary pregnancy urine and extracts of urine likewise proved to be effective agents. At the same time administration of

androgens to sexually active ground squirrels induced harmful effects (Wells, '35).

A consideration of these various findings and the facts with reference to the effects of t-propionate cited above leaves one searching for an apparently acceptable hypothesis that will bring the various findings into a common explanation. Without doubt too little is known of the metabolism of hormonal substances by the organism to warrant further speculation at the present time.

#### SUMMARY AND CONCLUSIONS

1. The pure chemical androgens testosterone and t-propionate induce secretory activity in the prostate gland and seminal vesicles of castrates that is indistinguishable from normal activity. These substances a) maintain normal histological conditions or repair damages in castrate males; b) induce precocious secretory activity; c) maintain the normal growth rate of accessory organs of reproduction in castrates during the most rapidly growing period of life and d) promote a normal prostate-seminal vesicle ratio when administered in dosages adequate to maintain normal growth of seminal vesicles; inadequate dosages for seminal vesicle growth maintenance naturally appear to favor prostate activity due to its lower threshold of response.

2. By weight t-propionate proved approximately four times as active as testosterone. Substitutional dosages for maintenance of growth rate of seminal vesicles was approximately three times dosage requirements for maintenance of histological normality.

3. T-propionate exhibited only slight prolongation of effectiveness after injection (approximately 3 days) as compared with testosterone, androsterone or ordinary castration of normal males.

4. T-propionate injections into rats castrated prepuberally and injected only after 6 months induced the animals to execute repeated and vigorous copulations, including ejaculations, within a period of 3 weeks. Seminal vesicle weight exceeded that of normal 7-month-old control males.

5. Testosterone and t-propionate cause grave retardation and injury to the testes of young normal males but this effect gradually declines with age. The propionate, especially, exhibited a reverse order of effects with increased dosage, 0.05 mg. often being more injurious than 1.0 mg. daily doses.

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# A REVIEW OF THE GOLGI APPARATUS

## PART III

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## 6. FUNCTION OF THE GOLGI APPARATUS

Undoubtedly the most important part of any discussion of the Golgi apparatus is that which deals with function. The value of all the information we have now, or may acquire later, of the form, structure, occurrence, origin, behavior, etc. of the apparatus is to be measured in terms of how much closer it brings us to an understanding of its function.

The omnipresence of the Golgi apparatus, in metazoon cells and, according to much evidence, in protozoan and plant cells also, indicates its participation in cellular activities to be of fundamental importance. Accordingly, it has been variously

<sup>1</sup> Parts I and II of this review appeared, respectively, in the March and April issues of *The Anatomical Record*.

suggested that the apparatus plays significant roles in cellular oxidations and reductions, syntheses and hydrolyses, condensations, catalyses, electrical polarizations and other similar vital phenomena.

In a series of papers Bounoure ('31, '37) advanced the theory that, in the developing egg of the frog, there is a special germinal cytoplasm (apparently Golgi apparatus) distinct from somatic cytoplasm and that as early as the blastula stage of development this 'cytoplasme germinal' acts as a germ cell determinant. The philosophical implications of this theory are so great that discussion must await confirmation of Bounoure's work.

Allen ('17), as interpreted by Bowen ('26 a), Hall and Nigrelli ('31), Weier ('30, '31, '32, '32 a, '33) and Dubosq and Grassé ('33) presented evidence that the Golgi apparatus may play a role in starch metabolism. The suggestion of Hall and Nigrelli is, however, of doubtful value since it rests upon the identification of the vacuome as the Golgi apparatus. While Allen, studying spermatogenesis in a plant, did not recognize the Golgi apparatus as such, he described a possible association of starch grains with a 'limosphere.' Wilson ('25) and Bowen ('26 a) agreed that Allen's limosphere is the Golgi zone. Weier, in a series of papers, advanced the suggestion, originally made and subsequently discarded by Bowen ('26 a), that the plastid of the plant cell corresponds to the Golgi zone and that it gives rise to the apical body in androcytes of certain mosses just as the acroblast (Golgi apparatus) gives rise to the acrosome in animal spermatocytes and spermatids (figs. 4, 8, and table 1). Hence it is not only possible but very probable that in plant cells, at any rate, the Golgi apparatus is concerned in carbohydrate metabolism. Since metabolic processes are largely dependent upon the oxidation of carbohydrates for their requisite energy, the potentialities inherent in this suggestion are apparent.

Very little attention has been paid to a possible role of the Golgi apparatus in cellular oxidation-reduction systems, although in 1912 Kingsbury suggested that mitochondria function in protoplasmic respiration—a suggestion which has been

accepted and amplified by Mayer, Rathery and Schaeffer ('14) and more recently by Ling, Liu and Lim ('28), Joyet-Lavergne ('35) and Bourne ('35). In view of the possible chemical similarity between mitochondria and Golgi apparatus, it might seem that the theory could be broadened to include the latter, but so far the evidence is very inconclusive. While mitochondria contain glutathione and vitamin A (Joyet-Lavergne, '35), vitamin C (Bourne, '35; Giroud, Leblond, Ratsimamanga and Rabinowicz, '35) and possibly a carotenoid pigment (Gatenby, '19) there is scant reason for suspecting the presence of a correspondingly adequate chemical basis for an oxidation-reduction system in the Golgi apparatus. Bourne suggested the possibility that glutathione and vitamin C may be present in Golgi materials, but he is uncertain about it. While the apparatus does not appear to contain any appreciable amount of iron, it is somehow intimately concerned in the intracellular behavior of certain iron compounds (Makarov, '33). So far the use of the peroxidase reaction, Unna's rongalit-methylene blue method for revealing oxygen stores, or other similar methods has disclosed nothing definite concerning a possible relationship between Golgi apparatus and cellular respiration. A continuation of such work as that of Needham and Needham ('27), Hirsch and Buchmann ('30), Nassonov ('30), Makarov ('33), Roskin and Semenoff ('33), Joyet-Lavergne ('35), Bourne ('35), Ries ('35) and Salazar ('36 a) may ultimately lead to significant information concerning this subject.

Lillie ('25), in emphasizing the importance of surface films in cellular systems, described the boundary of the Golgi apparatus as probably influencing the nature and rate of chemical reactions in the cell through variations in its physical (particularly electrical) and chemical properties. Similar influences, however, may be exerted by other surface films within the cell, such as those formed by vacuoles, fibrils, granules, chromosomes, and mitochondria (Cowdry, '26). The possible role played by the Golgi apparatus in a polyphasic system including chondriome, vacuome, a certain tannophilic, para-Golgi,

zone and the cytoplasmic substrate, was discussed by Salazar ('36 a).

While secretion is not generally considered a universal activity of living cells, it is the general consensus of current cytological opinion that the Golgi apparatus is intimately concerned in the processes of secretion. During the early years of the century many histologists objected that the reticulum was not universally present in cells. Now certain cytologists object because the apparatus is universally present. They point to the abundance of work demonstrating a Golgi apparatus participation in secretory processes, then object that Golgi material is found in all cells non-secretory as well as secretory. Although the relationship between Golgi apparatus and secretion was suspected by earlier workers (e.g., Fuchs, '02; Golgi, '09; Zawarzin, '09; Biondi, '11; Tello, '12; Cajal, '15; Deineka, '16; Hirschler, '18; Cowdry, '22; Hyman, '23), it was not given much attention by biologists until after the publication of the beautiful studies of Nasonov ('23 a, '26) and Bowen ('24 a, '26, '29). Even though the precise nature of this relation still remains unknown, the theory that it exists has received abundant confirmation in studies of practically all representative types of secretory cells. While such confirmatory data are as yet rather scant for certain glands, e.g., adrenal, hypophysis, parathyroids, gastric parietal cells; it is so unmistakable for the acinar portions of the pancreas, the salivary glands, thyroid, goblet cells, epididymis, stomach (chief and mucous cells), mammary gland and seminal vesicle that there can be no reasonable doubt about its general applicability to secretory processes. But the presence of the apparatus has been conclusively demonstrated in all types of animal cells both secretory and others; as Wilson ('25, p. 716) said:

. . . . it is hardly to be doubted that the Golgi apparatus plays an important part in secretion and related processes; but the presence of this structure in non-glandular cells such as nerve-cells, muscle-cells, connective-tissue-cells, and above all in the sperm-cells, indicates that it possesses a much broader significance.



The omnipresence of the apparatus does not necessarily tend to minimize its importance in secretory processes, since all cells contain intracellular enzymes. The manifestations of life are dependent upon the existence of numerous individual proteins synthesized by intracellular enzymes (Bergmann and Niemann, '37). It may well be in such secretory processes as those by which these proteins, and even the intracellular enzymes themselves, are produced that the Golgi apparatus is implicated. The increase in cellular metabolism known to occur during secretion, acquires additional cytological interest when correlated with the undeniable hypertrophy of the Golgi apparatus during the building up of secretion within the cell. Relating the apparatus to cellular metabolism in this manner is consistent with the size and complexity of the apparatus in adult nerve cells which have relatively high metabolic rates (Rau and Ludford, '25), but are not known to produce any external secretion (however, see Cowdry, '32; Nageotte, '32; Dornesco, '34, and Voinov, '34). In *Ascaris* eggs, according to Collier ('35), the Golgi bodies are small granules when the cell is quiescent, but enlarge as the metabolic rate increases.

If neutral red is really an indicator of enzyme activity, as claimed by Koehring, then Alexenko's ('30) descriptions of neutral red granules within the substance of the Golgi apparatus in the spinal ganglion cells of the fowl, and his claim that neutral red bodies are secreted by the Golgi apparatus in these cells, provides additional explanation of its presence in nerve cells. Although this particular part of Alexenko's work has not been confirmed by Beams ('31, '32) in his studies of the Golgi apparatus in ganglion cells of the grasshopper and rat, it deserves reinvestigation, particularly since Douglas, Duthie and Gatenby ('33) cast doubt upon the reliability of the technique employed by Beams. Beams described and illustrated smooth Golgi strands in spinal ganglion cells of the rat, very similar to those seen in figure 1. However, preparations of cervical spinal ganglion cells of the adult guinea pig (fig. 14) present quite a different picture. Here the strands of the Golgi reticulum contain small, sharply delineated, round

vesicles, at irregular intervals, thus giving a picture identical to that shown by Bowen in figures 8, 11 and 12 (reproduced here as fig. 9) of intestinal goblet cells and pancreatic cells of certain amphibians in his 1924 a discussion of the relation between the Golgi apparatus and secretory products. Since such minute vesicles in the Golgi substance are now generally recognized as indicating the first visible traces of secretory granules, this interpretation must be wrong or spinal ganglion cells (some of them at least) possess secretory activity. In this connection Brambell's ('23) belief in a secretory relationship between the Golgi apparatus, on the one hand, and the Nissl bodies and an oxyphile substance which he describes in nerve cells, on the other, is of interest.

Further light might be thrown on the possible relationship between Golgi apparatus and metabolism by a comparative study of the effect of dinitrophenol upon each in the same sample. Theoretically, inconspicuous apparatuses in cells with low metabolic rates would be expected to show a certain amount of hypertrophy after stimulation. Other approaches to the study of the apparatus in cells of varying metabolic conditions could easily be devised. For example, comparative metabolic and cytologic studies might be made of cells following exposure to alcohol, cyanide, cold, heat, atmospheres of pure hydrogen or of oxygen, etc. Or again the effect of thyroidectomy, hypophysectomy, inanition, devascularization, stimulation of mitosis, regeneration, compensatory hypertrophy, suffocation, etc., upon the cell as determined by simultaneous metabolic and cytologic studies might readily be made. A few of the many papers suggesting specific approaches to this problem are those of Baillif ('37), Bourne ('35), Byerly ('26), Eastlick ('36), Guyer and Claus ('37), Ludford ('35, '36), Nassonov ('30) and Ries ('37).

Viewing secretion from another angle, there seems to be no good reason for not considering all cells as secretory (excretory) in the sense that they all give off waste products. The cytologist may consider himself justified in thinking of all secretions as being waste products of the cells producing them,

no matter how useful they may be to the organism as a whole. In this connection the point of view of Koehring ('30) as given in the following quotation, is pertinent: "Each tissue is not only specific in its hydrolytic and synthetic complex, but it is specific in its nutriment selection as well as its secretion. The endocrine system thus comprises every tissue in the body." If it be true that the Golgi apparatus is an essential part of all secretory mechanisms, it would be inexplicable not to find it in all living cells. Essentially the same idea was expressed by Subramaniam ('34, '36, '37) who suggested that "in ordinary cells, metabolism is carried on by enzymes secreted by the Golgi. Naturally in specialized cells, in addition to their ordinary function, these enzymes also synthesize products such as yolk, fat, mucous, serous, lipid droplets, etc."

All direct evidence in favor of a relationship between Golgi apparatus and secretion is, of course, derived from studies of certain gland cells in which secretory activity can be detected cytologically. Some of these studies indicate that the apparatus is the actual synthetic center for secretory antecedents. It is even claimed that active enzymes may be demonstrated there. According to other studies, the apparatus is actually transformed into secretory products. A great deal of work strongly suggests that the Golgi apparatus neither synthesizes secretory substances nor is transformed directly into them; but that it acts as a condensation membrane, for the concentration, into droplets or granules, of products elaborated elsewhere and diffused into the cytoplasm. These elaborated products may be lipoids, yolk, bile constituents, enzymes, hormones, or almost any other formed substance; even mitochondria may be concentrated out of the cytoplasm and given back to it as filaments, rods, or granules, according to some observations.

The classic evidence indicating that the Golgi apparatus is the center of secretory synthesis is the manner of formation of the acrosome (fig. 4). This evidence is so clear-cut in many instances that the only way of getting around it is by claiming, with the upholders of the vacuome hypothesis, that the dictyosomes of male germ cells do not represent Golgi material, but,

as stated by Wilson and Pollister ('37), this claim can no longer be supported in the face of conclusive evidence that "there is no substantial difference between the Golgi bodies of the germ cells and those of other tissue cells" (Hirschler, '18; Bowen, '26 a; Monné, '30; Sembrat, '30; etc.).

The relation of the Golgi apparatus to the development of the acrosome has been so thoroughly studied that it has already become incorporated into standard textbooks of cytology (Sharp, '34), histology (Maximow and Bloom, '30) and embryology (Arey, '34). Consequently, no more than a brief statement of the process need be included here. In the spermatids the Golgi material, usually in the form of separate units, is found ordinarily as a cortical layer at the periphery of the idiozome. This combination of Golgi material and idiozome is called an acroblast. Sometimes the Golgi bodies appear to have fused to form an incomplete cortical shell around the idiozome (Hemiptera) while in still other cases each Golgi body has its own separate bit of idiozomal substance attached to it (Lepidoptera and some Orthoptera). No matter whether the acroblast is of the single or multiple type the formation of the acrosome always seems to take place by one of two methods. In the majority of instances a vesicle appears in the idiozome (several vesicles in multiple acroblasts); a granule then makes its appearance within the vesicle. As the vesicle and its contained granule enlarge, the entire acroblast may migrate around the nucleus to the tip of the spermatid where the acrosome is deposited in situ; or the acrosome may be deposited on the nuclear membrane and subsequently move to the tip, perhaps through the rotation of the nucleus. In a few animals (Mollusca and some Orthoptera) the acrosomal granule is formed with no indication of an enclosing vesicle. The subsequent behavior of the granule is similar to that exhibited by the vesicular type of acrosome. No matter how the acrosome may be formed it resembles any other secretory granule in that after once losing its connection with the Golgi apparatus (acroblast) it remains independent of it. For detailed descriptions of acrosome formation see especially the



various papers of Bowen and Gatenby. The subject is included here only as the outstanding illustration of the participation of Golgi material in secretory activities.

Severinghaus ('33 a), in extensive studies on the sheep thyroid, described and illustrated a relationship between the formation of intracellular colloid vesicles and Golgi material which is similar to and almost as striking as the relationship between acroblast and acrosome in male germ cells. It would indicate that the dictyosomes and the Golgi apparatus of somatic cells are homologous structures.

The function of the Golgi apparatus in the egg is not nearly so certain as in the sperm. While the majority of workers find evidence that fatty yolk is of Golgi origin there remains a small minority whose work suggests that the Golgi apparatus is just one part of the fatty yolk producing mechanism in egg cells. Only a few investigators trace albuminous yolk to a Golgi origin. Subramaniam ('35) was of the opinion that the Golgi apparatus is concerned in the production of both lipoidal and albuminous nutritive bodies. The confusing literature of yolk formation was reviewed by Gatenby and Woodger ('20), Bowen ('26 a) and MacBride and Hewer ('31). Little of a more definite character can be stated for the real role of the Golgi apparatus in yolk formation until the use of new technical methods, or of more useful modifications of existing ones, harmonizes or explains the contradictory observations upon which our present confusion is based. That the Golgi apparatus is concerned in the synthesis of fatty substances in somatic cells is strongly indicated by the work of Cramer and Ludford ('25) describing the synthesis of fat by intestinal cells during fat absorption. Equally convincing, however, is the work of Liu ('30) who found that the mitochondria of the intestinal absorbing cells undergo very striking alterations during fat absorption. In this connection the transformation of the apparatus into fat in tissue-cultured cells (Ludford, '27; Richardson, '34; Macdougald, '35; and Macdougald and Gatenby, '35) should be recalled, but also the

finding of Hill ('36) and Macdougald ('37) that under optimum conditions of cultivation the transformation does not occur.

The classical work of Bensley ('11) on the nature of prozymogen granules in pancreatic cells and the more recent work of Nassonov ('23 a, '24), Bowen ('24 a, '26), Morelle ('25), Gatenby ('31 a) and others, strongly suggests that, in these cells at least, the work of secretory synthesis is carried on by the Golgi apparatus itself; the smallest prozymogen granules appearing not only in contact with Golgi substance but actually within it. While the figures of Morelle and Gatenby are convincingly clear, it must be pointed out that not all modern workers are in accord. Beams ('30), for example, never found neutral red granules (vacuome of Parat and prozymogen of Bensley) fused with the strands of the Golgi apparatus.

Twenty years before the discovery of the Golgi apparatus, Nussbaum (1878) proposed that the enzyme producing activity of glandular cells could be correlated with their capacity to blacken with osmic acid. The more recent proposal, that the osmiophilic Golgi apparatus is a locus of enzyme action, is reminiscent of Nussbaum's suggestion, although, unlike the latter, it does not depend entirely upon the capacity to blacken with osmic acid. Bowen ('24 a), who did so much to develop this idea, expressed it in the following words:

. . . . we have in the relation of the Golgi apparatus to secretory phenomena a distinct indication of its relation to processes of chemical synthesis in the cell, and from this it is only a step to the conception of the Golgi apparatus as a great intracellular center of chemical synthesis or enzyme formation.

While the brilliant investigations undertaken by Bowen in developing and testing his theory were cut short by an untimely death, the able presentation of his views had won them almost immediate acceptance by the majority of biologists. That this conception is applicable to plant cells as well as to those of animals is suggested by the words of Weier ('32, p. 668) who, after presenting evidence of a correspondence between Golgi zone and plastid (fig. 8 and table 1), wrote:

It has been shown that in addition to chlorophyll and sunlight some additional cytoplasmic factor, in all likelihood of an enzymatic nature, is necessary for the production of starch. May it not be, then, that at least one of the direct products of the plastid cytoplasm is an enzyme? If this should be the case there would be a harmony of structure and function between the animal and plant cell. Both possess, in addition to the already well established structures, regions of cytoplasm which upon fixation present corresponding plate-work forms. During meiosis and spermatogenesis these specialized regions of cytoplasm have a very similar history. In function they both elaborate enzymes. The greatest difference in the two would lie in the fact that through some photochemical reaction, as yet not understood, the plastid is enabled to utilize the energy of the sunlight to build up the simple sugars.

Within the last few years, however, there has been a growing tendency to transfer the conception of synthetic properties from Golgi apparatus to other cytoplasmic constituents, especially the mitochondria, and to conceive of the Golgi apparatus as a condensation membrane serving to concentrate synthesized substances into droplets. In the words of Ludford ('28), who based his opinion not only upon the results of his own investigations but also upon the work of Cowdry ('26), Nasonov ('26) and Horning and Petrie ('27):

At the mitochondrial-cytoplasmic surface syntheses by enzymes occur. The resulting products continually diffuse into the cytoplasm, preventing an accumulation at the surface of the mitochondria, which would inhibit further synthesis. At the surface of the Golgi apparatus the elaborated products are concentrated into droplets, as a preliminary to their elimination.

More recently Koehring ('30) has taken the idea of the synthetic properties of mitochondria and applied it universally to animal cells. She, however, considered the Golgi apparatus to be a part of the mitochondrial system, the synthetic properties of which appear to depend upon lipoidal composition. Similar views were held by Ling, Liu and Lim ('28), Ma ('28, '28 a), Hirsch ('31, '32), Duthie ('33, '34), and Siang Hsu ('35).

Much of the evidence favoring the view that the Golgi apparatus acts as a condensation membrane comes from such studies as those of Jasswain ('25 a), Nassonov ('26, '27), Makarov ('26), Cramer and Ludford ('26), Glasunov ('28), Rumjantzew ('29), Ludford ('28), Chlopin ('27, '30) and Weatherford ('32) on the elimination of trypan blue by kidney and liver cells. This work indicates that acid vital dyes entering liver and kidney cells in a diffuse and invisible condition become segregated and condensed into droplets by the Golgi apparatus before being eliminated into the urinary tubules or the bile canaliculi. Since, thanks to the important work of von Möllendorff ('26), it is now well known that acid vital dyes, unlike basic ones, do not stain preformed bodies within cells, but, after absorption by certain cells, become deposited in induced vacuoles, there seems no escape from the conclusion that the reaction of these cells to such dyes is an expression of a normal secretory mechanism, and since the newly-formed droplets and the Golgi apparatus have been demonstrated side by side in the same cells (fig. 6) and in the same topographical relations as true secretory droplets and Golgi apparatus, one must again conclude that the topographical appearance of secretory droplets, as evinced in the reaction of liver and kidney cells to acid vital dyes, suggests nothing so much as a segregating and condensing function for the Golgi substances. Several authors have not hesitated to apply these observations to the secretion of bile pigments and salts by the liver. Although Hirsch ('31) agreed that the 'Golgi field' is a center of condensation, and describes the ripening of zymogen granules in the pancreas in connection with Golgi colloids, he stated that pre-zymogen granules appear first in topographical connection with mitochondria, a relationship which Duthie ('34) found in the Harderian gland of the rat.

If it be true that the Golgi apparatus functions as a condensation membrane, the variable form which it exhibits would appear to be of small consequence. It might be a simple or a multiple sac, plate, cup, rodlet or granule without having its



function as a condensing mechanism seriously affected, except quantitatively, since its efficiency would be partly subject to the extent of its cytoplasmic surface.

The claim has been made frequently that the Golgi apparatus is transformed directly into secretory substances. If this be true it must either be reformed *de novo* or arise from portions of the original apparatus remaining unchanged. The often repeated observations that the Golgi apparatus definitely enlarges during secretory activity far outweighs in significance the scanty and uncertain evidence that it ever actually changes into secretory granules.

If the Golgi apparatus is to be considered as an indicator not only of the production of substances destined for exportation, but also of the manufacture of living protoplasm or its various intermediary substances, the question of the secretory significance of a polarized apparatus loses much of its interest. Of course, the participation of Golgi material in the more vital activities of cells need not preclude its taking part in secondary activities, such as the production of external secretions, but it would tend to minimize, rather than to accentuate, the likelihood that changes in the polarity of the apparatus parallel changes in the direction of secretion, as Cowdry has postulated for the thyroid. However, until our knowledge of the Golgi apparatus is considerably more extensive than at present, it is unwise to interpret its behavior along certain limited lines. On the basis of studies on the liver Pollister ('32) went so far as to exclude endocrine activities, and limited the apparatus to a participation in exocrine secretory processes. The present tendency to search for correlations between Golgi apparatus and the production of external secretions may blind us to its probable broader significance. This danger was fully realized by Ma ('28), Beams and King ('32) and an increasing number of other writers who stressed the belief that neither the Golgi apparatus nor any one of the other cytoplasmic constituents should be thought of as the sole synthetic center of the cell but rather that they all belong to a complex system which is itself the functional unit of the cell.

We know of no more fitting conclusion to this review than the following words from Wilson ('31):

The spectacle of the cell at work is one that we view with a wonder that bears witness to our ignorance concerning what lies behind the show. It is as if the performance were carried through with perfect understanding and competence, but with no intention of explaining the trick. It is a tantalizing but profoundly interesting display and one that places before our eyes an alluring field for further exploration by experimental research.

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<sup>2</sup>1. Journal abbreviations are those used by the Quarterly Cumulative Index Medicus.

2. References cited in the text, but not included in this bibliography, may be found in one of the following papers: Bowen ('28, '29), Hertwig ('29), Gatenby ('31 a), Nahm ('33).

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# THE EFFECT OF MALE HORMONE SUBSTANCE UPON THE TESTES AND UPON SPERMATOGENESIS

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ONE PLATE (FIVE FIGURES)

Male hormonal substances have been reported to maintain (Walsh, Cuyler and McCullagh, '34; Nelson and Gallagher, '36; Engle, Moore, McCullagh, Nelson, Greep and Okkels, '37) but not to initiate (Nelson and Gallagher, '36) spermatogenesis in the hypophysectomized rat. This communication is a study of the effect of synthetic male hormones on the scrotal and cryptorchid testes of normal and hypophysectomized rats.

## MATERIALS AND METHODS

Forty-one rats of an inbred strain, weighing from 110 to 150 gm., were hypophysectomized and separated into a control group and a group which received daily injections of 500 gamma of testosterone acetate<sup>1</sup> or testosterone propionate.<sup>1</sup> One tenth of a cubic centimeter of peanut oil was used for each injection since the amount and type of oil influence the absorption of the hormone (Parkes, '36).

In each rat at the time of hypophysectomy, one testis was confined in the abdomen, the other in the scrotum by narrowing the inguinal canal with sutures, in a manner described previously (Leonard and Hamilton, '37). Thus, migration of

<sup>1</sup> Testosterone acetate and testosterone propionate were furnished by Ciba Company under the trade name of Perandren.

testes between abdomen and scrotum was avoided. To minimize unrecognized changes in temperature which might influence the testicle, all animals were kept in a constant temperature chamber at 82 to 84°F., since the environmental temperature readily affects the body temperature of rats (Hamilton, '37 a), especially that of hypophysectomized rats. Injections were begun in eighteen of the animals at the time of operation, in four other animals 9, 16, 24, and 51 days after hypophysectomy. A pair or more of control and experimental animals were killed at intervals varying from 1 to 44 days. Body and organ weights were taken at autopsy and the testes prepared for microscopic study.

A further set of twelve male rats were divided into litter-mate control and injected groups, those in the injected group receiving 500 gamma of testosterone acetate daily from the time of weaning until all animals were sacrificed at the age of 4 months.

#### RESULTS

As shown in tables 1 and 2 hypophysectomized rats receiving testosterone compounds immediately after the operation had not only heavier scrotal testes but also heavier abdominal testes than those of hypophysectomized, uninjected rats.

##### *A. Maintenance of spermatogenesis*

*Effect on scrotal testes.* The scrotal testes of the hormone-injected animals 13 to 44 days after treatment averaged 0.62 gm., or 94% heavier than those of the control animals (0.32 gm.). The difference between the scrotal testes of injected and control animals became pronounced by 14 days and continued to be striking at 44 days, the longest period studied (figs. 1 and 2). Histologically, the tubules of injected animals remained large in size and contained cells in all stages of spermatogenesis.

During the first week following hypophysectomy only slight differences could be seen between the scrotal testes of control and injected animals, but by the end of the second week, concomitant with the changes evidenced grossly in differences in weight, considerable histological dissimilarity was observed.



TABLE 1

*Weight of scrotal and abdominal testes of androgen-injected and control rats. All animals were hypophysectomized. Those marked \* were injected daily with 500 gamma of testosterone acetate or propionate beginning the day of operation. Note the heavier weight of both scrotal and abdominal testes in injected animals*

| Animal | Body weight (in grams) |         | Number of days<br>between<br>hypophysectomy<br>and autopsy | Testis weight (in grams) |         |
|--------|------------------------|---------|------------------------------------------------------------|--------------------------|---------|
|        | Operation              | Autopsy |                                                            | Abdominal                | Scrotal |
| * 28   | 111                    | 102     | 1                                                          | 0.792                    | 0.829   |
| 33     | 133                    | 116     | 1                                                          | 0.780                    | 0.945   |
| * 49   | 151                    | 128     | 2                                                          | 0.751                    | 1.089   |
| 26     | 142                    | 118     | 2                                                          | 0.721                    | 1.034   |
| * 29   | 117                    | 109     | 6                                                          | 0.397                    | 0.8860  |
| 38     | 150                    | 98      | 6                                                          | 0.347                    | 0.9516  |
| * 39   | 138                    | 114     | 6                                                          | 0.337                    | 0.8186  |
| 47     | 151                    | 127     | 6                                                          | 0.348                    | 0.9390  |
| 44     | 148                    | 117     | 7                                                          | 0.4087                   | 0.8647  |
| * 5    | 117                    | 92      | 9                                                          | 0.194                    | 0.478   |
| 13     | 116                    | 104     | 9                                                          | 0.177                    | 0.344   |
| * 23   | 118                    | 100     | 12                                                         | 0.130                    | 0.279   |
| 10     | 119                    | 112     | 12                                                         | 0.149                    | 0.290   |
| * 43   | 148                    | 139     | 13                                                         | 0.267                    | 1.076   |
| 41     | 150                    | 124     | 14                                                         | 0.158                    | 0.648   |
| * 46   | 135                    | 123     | 14                                                         | 0.226                    | 0.8464  |
| * 19   | 120                    | 98      | 15                                                         | 0.265                    | 0.813   |
| 18     | 120                    | 106     | 15                                                         | 0.127                    | 0.673   |
| * 14   | 116                    | 111     | 15                                                         | 0.169                    | 0.558   |
| 4      | 116                    | 111     | 15                                                         | 0.140                    | 0.330   |
| * 20   | 128                    | 118     | 18                                                         | 0.198                    | 0.874   |
| 11     | 125                    | 113     | 18                                                         | 0.139                    | 0.252   |
| * 35   | 134                    | 84      | 20                                                         | 0.2265                   | 0.6837  |
| * 3    | 125                    | 129     | 21                                                         | 0.163                    | 0.432   |
| 24     | 122                    | 98      | 21                                                         | 0.117                    | 0.164   |
| * 50   | 157                    | 130     | 27                                                         | 0.193                    | 0.783   |
| 36     | 141                    | 108     | 27                                                         | 0.115                    | 0.187   |
| * 30   | 137                    | 125     | 28                                                         | 0.161                    | 0.724   |
| * 6    | 129                    | 132     | 35                                                         | 0.132                    | 0.326   |
| 9      | 131                    | 122     | 35                                                         | 0.122                    | 0.172   |
| * 32   | ...                    | 112     | 44                                                         | 0.151                    | 0.354   |
| 31     | ...                    | 104     | 44                                                         | 0.114                    | 0.134   |

In the control animals there were few, and in some cases no spermatozoa, although some sperm heads and spermatids were present. In injected animals many tubules were apparently normal. By the beginning of the third week the tubules of control rats presented no sperm and only few spermatids. The tubules of injected rats maintained a rather normal appearance, although mitoses were much fewer than previously. By the fourth week the tubules of control animals were in an advanced degenerate condition (Leonard and Hamilton, '37), and lacked spermatozoa and spermatids; mitoses were uncommon. At the end of the fifth and sixth weeks, cellular atrophy and shrinking of the tubules had proceeded still further in the control animals. In injected rats

TABLE 2

*The average weight of testes from control and from androgen-injected rats, which were sacrificed 13 to 44 days post-operatively, shows the greater weight of both scrotal and cryptorchid testes in injected animals. Body weight was not a factor since the injected animals in the series averaged 118 gm., the control animals 111 gm.*

| <i>Position of testis</i> | <i>Testicle weight in androgen-injected rats (in grams)</i> | <i>Testicle weight in control rats (in grams)</i> | <i>Percentage difference</i> |
|---------------------------|-------------------------------------------------------------|---------------------------------------------------|------------------------------|
| Scrotal                   | 0.62                                                        | 0.32                                              | 94                           |
| Abdominal                 | 0.182                                                       | 0.126                                             | 44                           |

some changes appeared in that the spermatids tended to clump (fig. 3); mitoses, although more frequent than in control rats, were not as common as previously. The lumina were empty, and cells in the periphery of the tubules were more in a degenerative state.

*Effect on abdominal testes.* The abdominal testes of hormone-injected animals 13 to 44 days post-operatively averaged 0.182 gm., or 44% heavier than those of control animals (0.1265 gm.). Histologically, however, tubules of the injected rats presented a more degenerate condition than those of control animals, for although the tubules remained large in size the cells of the seminiferous epithelium underwent cytolysis more rapidly and completely. But very little difference was observed in the abdominal testes between injected and control

animals the first week following hypophysectomy, although it seemed that those of the injected rats were perhaps slightly less degenerate. By the second week the tubules from injected animals appeared to be somewhat larger. In the animals sacrificed 15 to 18 days subsequent to hypophysectomy the greatest difference was observed microscopically between the testes of injected and control animals. In comparison with tubules of control animals, those of injected animals were larger and had fewer layers of cells within the tubules, larger empty lumina, a lighter staining reaction, and an almost complete lack of spermatocytes (figs. 4 and 5). From the third week after hypophysectomy to the termination of the experiments at the end of the sixth week, those differences between the testes of control and experimental animals became less pronounced.

Since the body weights at autopsy of the control animals averaged 111 gm., as compared to 118 gm. for injected animals, the testicular differences could not be attributed to the bodily condition of the animals. Testosterone compounds did not stimulate or maintain the interstitial cells in either scrotal or abdominal testes of any hypophysectomized animal.

### *B. Failure of initiation of spermatogenesis*

When injections were postponed until 9, 16, 24, or 51 days after hypophysectomy, well-developed tubules were not obtained. This result was checked in two animals by removing one testis before injection of testosterone compounds. No spermatogenesis was ascertained in either case, although in some rats the testes were again seen to occupy a scrotal site.

Further proof that spermatogenesis was not stimulated anew was seen in that six male rats, injected daily with 500 gamma of testosterone acetate from weaning until the age of 4 months, showed less tubular development than did five control uninjected litter mates. In injected animals the interstitial tissue was very much reduced and spermatozoa lacking. Litter-mate controls exhibited well-developed interstitial tissue and spermatozoa. Similar results have been reported by Moore and Price ('37).

Perhaps worthy of note is the confirmation of facts set forth in an earlier study (Leonard and Hamilton, '37) that degeneration of the scrotal testes may not be marked histologically until more than a week after hypophysectomy, and that scrotal and abdominal testes of uninjected hypophysectomized animals approach a similar condition after 3 weeks.

#### DISCUSSION

The above results show that testosterone compounds maintain spermatogenesis in the hypophysectomized rat similar to the action of gonadotropic substances (Smith and Leonard, '34; Smith, Engle and Tyndale, '34). Not only do these androgens exert an effect on testes located in the scrotum, but also directly upon cryptorchid testes of the hypophysectomized rat. The maintenance of spermatogenesis connotes stimulation of cell division in existent spermatogonia, and it appeared that mitoses in the scrotal testes were much more numerous in injected animals than in control animals. Check should be made, however, upon the relative number of mitoses found by the colchicine technique (Allen et al., '37) when androgenic substances are administered.

The obvious question arises as to how male hormones maintain spermatogenic activity in scrotally retained testes of the hypophysectomized rat. It has been shown that male hormones act to develop and maintain the scrotum and to promote a scrotal position of the testes (Hamilton, '36). The importance of the scrotum as a thermoregulator is thoroughly established, but the role played by androgens in maintaining spermatogenesis through scrotal normality (Hamilton, '36) cannot be extended as has been suggested (Cutuly, McCullagh and Cutuly, '37) to possibly largely explain the phenomena of tubular maintenance. Such a simple explanation is not an entire answer for 1) retention of the testes of the hypophysectomized rat in the scrotum will not in itself suffice to maintain spermatogenesis (Leonard and Hamilton, '37); 2) as shown by the data of this communication, androgens are capable of exerting an influence on cryptorchid testes, in which



condition the status of the scrotum is entirely irrelevant; 3) in the normal animal high levels of androgenic material, through suppression of pituitary activity (Wolfe and Hamilton, '37 a and b; Hamilton and Wolfe, '38; Moore and Price, '37) might be expected to have an inhibitory influence upon the testes; and in fact such androgen inhibition of the pituitary as shown above can be sufficient to prevent normal development of the testis; 4) the degree of spermatogenesis is not necessarily correlated with the degree of scrotal development (Nelson and Merckel, '37).

In addition to the maintenance of functional scrotal testes, there is also an effect on cryptorchid testes exerted by testosterone compounds. The cryptorchid testes of injected animals are heavier and have larger tubules, but after a week or more the cells lining the tubules are more degenerate in appearance than those of control hypophysectomized animals which were not injected with androgenic material.

The effect of androgens on the tubular epithelium of scrotal testes may be due in part to scrotal normality, but a different explanation must be sought for the effect upon the abdominal testes and it becomes apparent that, in addition to an indirect effect through development of the scrotum, androgens also directly affect tubular tissue. Possible mechanisms that may be implicated in the effect of androgenic material on cryptorchid testes are as follows:

1. A direct stimulation of seminiferous epithelium. If this were so, it would aid in accounting for maintenance of the scrotal testes, and stimulation of the cryptorchid testes in their excessively warm location might be expected to induce the earlier necrosis found in injected animals.

2. No matter whether produced by stimulation of seminiferous epithelium in the excessively warm, hence injurious abdominal temperature, or by some other means, there is a more advanced cellular destruction produced in the cryptorchid testes of hypophysectomized animals when they are injected with androgenic material. It is possible that an increased collection of fluid may accompany the necrosis and thus, at least in part account for the greater tubular size and testicular weight than in controls.

3. Also possible is a direct maintenance of the size of the tubule wall, thereby resulting in a greater size of the tubules and a heavier testicular weight.

4. Finally, it may be that the effect is mediated via a change in the blood supply rather than directly upon the tubular tissue.

The action of male hormone in maintaining testicular activity in the hypophysectomized rat and in the failure of this hormone to return the testes to normal in the postponed treatment resembles the action of pregnancy urine extract on the testes of the hypophysectomized rat. Since pregnancy urine acts for the most part on the interstitial cells, it seems quite possible that androgenic material produced by the pregnancy urine may be an active agent in maintaining the testes in this case. A similar suggestion has been made that the descent of the testes produced by pregnancy urine extracts might be at least in part an indirect action through the production of male hormones, and that the androgenic substances were in part the agents actually responsible (Hamilton, '37 b).

The effect of androgens in the maintenance of an existent spermatogenesis, in the production of testicular descent and in the induction of penile erections (Hamilton, '37 c) and of genital development in humans (Hamilton, '37 d) will doubtless suggest their use in the treatment of certain cases of sterility. But application to man of facts observed in rats and monkeys must await proof. Too, it would seem optimistic to expect a gametokinetic stimulation other than that resulting from stimulation of an already existent spermatogenesis and from placement of the testes in a functional, well-developed scrotum. Indeed, since androgenic materials decrease the size and stimulatory powers of the pituitary, their substitution over long periods of time for bodily produced or injected gametokinetic substance would appear contra-indicated save in the absence of normal testicular secretions. Perhaps a conservative expectation of the usefulness of androgens in sterility would be 1) in stimulation of existent spermatogenesis, 2) as a possible aid following administration of a gametokinetic substance, 3) in certain cases, where there is lacking

a) testicular descent and/or b) a functional scrotum, 4) in inducing penile erections where this capacity is limited, and 5) in sexually underdeveloped individuals where growth of the sexual organs could be obtained.

These effects on the testis necessitate remodelling of existent theories of hypophyseal-gonad interrelationships. The conception of Moore and Price ('32) among others that administration of gonadal hormone has only an injurious effect on the gonad mediated through the pituitary is not wholly true with regard to the adult and constant-breeding male animal. True, histologic and physiologic studies of pituitaries in animals which received testosterone compounds reveal a decreased gonadotropic activity, yet such suppression of gonadotropic activity is partially counterbalanced by an action maintaining spermatogenesis. Thus, any theory of gonad-pituitary relationship must include an androgen-on-the-testis component in addition to the androgen-pituitary-testis balance. Species difference must also be remembered as exemplified by the precocious spermatogenesis produced in the ground squirrel by androgenic material (Wells and Moore, '36).

#### SUMMARY

Forty-one rats of the same age and inbred strain were hypophysectomized, and in each rat one testis was confined by ligature in the scrotum, the other in the abdomen. Half the group received injections of testosterone acetate or propionate; the other half of the group served as controls. Injected and comparable control animals were sacrificed at intervals from 1 to 44 days after hypophysectomy. Other rats were injected each with testosterone acetate or propionate from weaning until they were 4 months old. The following conclusions are suggested with regard to the rat:

1. These testosterone compounds maintain spermatogenesis in scrotal testes of hypophysectomized rats, but do not stimulate spermatogenesis a) precociously or b) after post-hypophysectomy degeneration has ensued.

2. Testosterone acetate and propionate affect tubular tissue, not only when the testis is in the scrotum but also when the testis is in the abdomen. In comparison with control animals the effect on cryptorchid testes consists of a greater testicular weight, larger tubules and a more rapid degeneration of tubular contents.

3. This effect on cryptorchid testes indicates that androgens have their effect not only indirectly by scrotal normality and placement of the testes with the scrotum, but also directly upon tubular tissue.

Discussion is given of the possible mechanism of this effect on cryptorchid testes, the usefulness and limitations of androgens in the treatment of sterility, and the pertinence to the conception of gonad-hypophyseal interrelationships of the finding that androgens have a direct effect on seminiferous epithelium.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Section of the scrotal testis of control (no androgen injections) rat no. 24, taken 21 days after hypophysectomy.

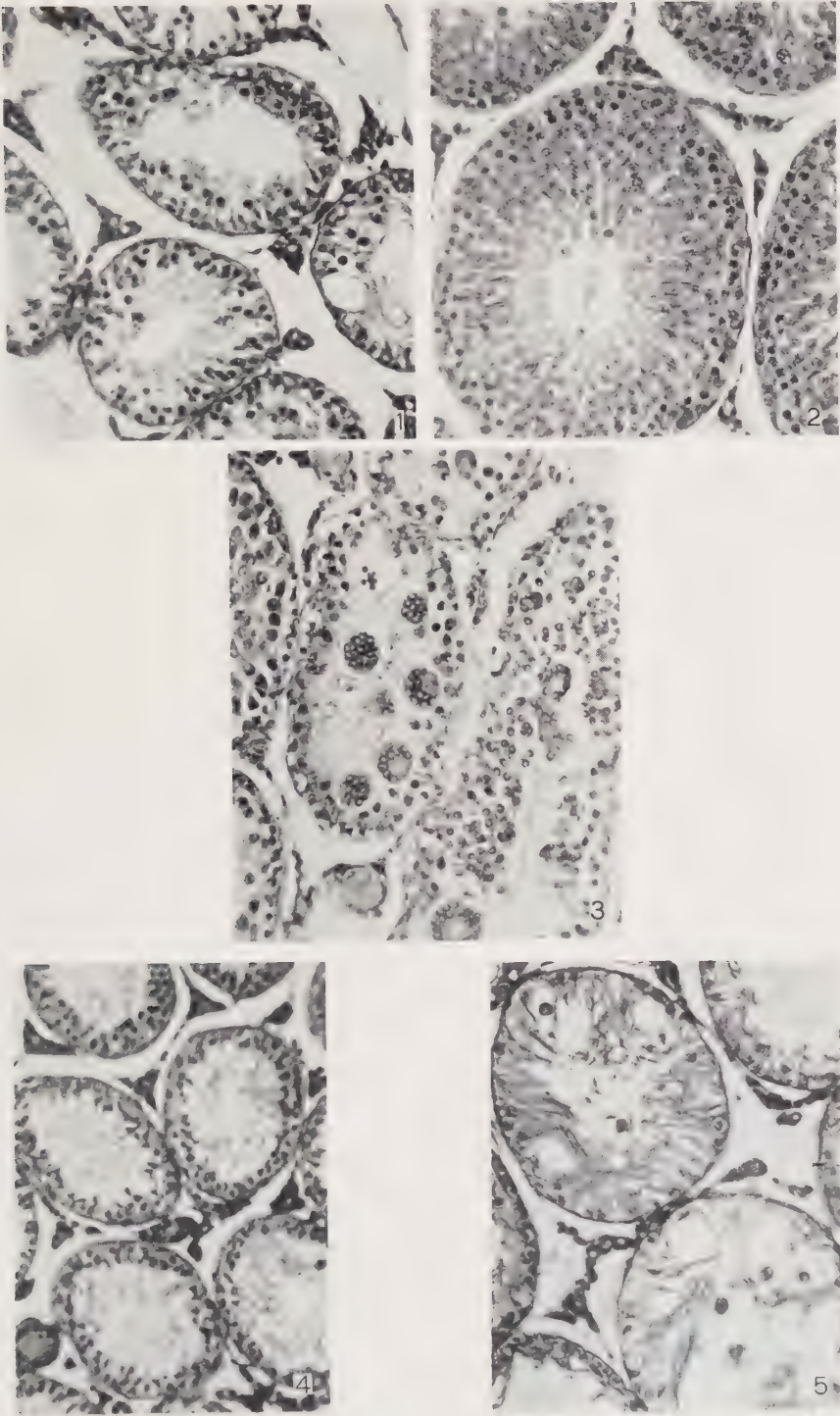
2 Section of the scrotal testis of androgen-injected rat no. 3, taken 21 days after hypophysectomy.

3 Section of the scrotal testis of androgen-injected rat no. 32, taken 44 days after hypophysectomy.

4 Section of the abdominal testis of control rat no. 18, taken 15 days after hypophysectomy.

5 Section of the abdominal testis of androgen-injected rat no. 19, taken 15 days after hypophysectomy.

Testosterone acetate and testosterone propionate exerted an effect on the testis whether it be in the scrotum or in the abdomen. Hence, the influence of these androgenic compounds was not solely through maintenance of scrotal normality. In comparison with litter-mate control animals these androgens favored degeneration of tubular contents in the cryptorchid testis, maintenance of spermatogenesis in the scrotal testes and a heavier weight and larger tubule diameter in both the cryptorchid and the scrotal testis.







## BOOK REVIEW

A MONOGRAPH ON VEINS. By Kenneth J. Franklin, D.M., M.R.C.P., tutor and lecturer in physiology, Oriel College; University demonstrator of pharmacology; assistant director of the Nuffield Institute for Medical Research, Oxford. Price, \$6.00. 420 pp., with 45 illustrations. Springfield, Ill. and Baltimore. Charles C. Thomas, publisher, 1937.

As the boundless mass of science is disrupted into smaller and smaller bits for intensive inquiry, the implications of losing sight of the neighbor's findings because of preoccupation with one's own—especially the possibility of their oblivion, or of missing the chance of discovering a greater truth which the fitting together of the bits might disclose—compel in the same degree attempts at integration. During recent years there is manifest an increasing drift toward the correlation of the total knowledge of a circumscribed subject from the points of view of its different facets. 'A Monograph on Veins' represents such an endeavor—not by an onlooker, but by one who has been in the thick of the research since 1924, and who can build solidly on his own work. Doctor Franklin says more modestly that the "object in writing the book was to make available to others a somewhat recondite literature, which only a specialist could hope to summarize but which has, nevertheless, very definite bearings upon physiological, pathological, and clinical problems." Because his focus is through the experimental approach, the morphological facet may seem at times to shine less brightly, yet it illuminates adequately the portrayal of the behavior of the veins.

Of the only two previous summaries of the subject, namely the work of Klothilde Gollwitzer-Meier ('32) in German, and that of M. L. Waterman ('33) in Dutch, the former influenced Franklin in the presentation of the material, for as he says "the more recent re-orientation of ideas about the venous system and its physiology has been in large measure due to Professor Gollwitzer-Meier and to Professor Alfred Fleisch." Of his friends, Professors Ebbecke, Philipp Stöhr, Bürger, Alfred Jäger, Robert Janker, Naegeli, and others at the University of Bonn where he lectured in May, 1935, Franklin speaks with appreciation for the stimulus and help received.

The frontispiece, a portrait of William Harvey, and the words of Goethe: "In dem Vergangnen lebt das Tüchtige, verewigt sich in schöner That," which preface the historical chapter, stamp the dignity of the book and invite to its perusal. A survey, replete though compressed in eight pages, traces the faltering steps to the knowledge of the circulatory system, from the times of Alcmaeon of Croton in the 6th century, B.C., to the times of Harvey. By taking for granted the story of Harvey's discovery, and quoting but the gist of the only conversation Harvey had with Robert Boyle that it was a consideration of the valves in the veins which led him to think of a circulation, the author avoids the charge of redundancy and proves his good taste.

Chapter II, briefly reciting the parts played respectively by the heart, arteries, capillaries and veins in the circulation, emphasizes that the last have not yet received the measure of attention which is due them, for the venous system is not merely the passive return route of the circuit, but participates markedly in other functions.

Deciding that he could not cope successfully with the task of writing the chapter on embryology of the veins, Doctor Franklin prevailed upon his friend, Keith Richardson, of University College, London, to provide a summary; this is based largely on the comprehensive investigations of American anatomists—F. T. Lewis, Huntington, McClure, Reagan, Butler, Streeter, and others.

The motif in the fourth chapter, which deals with the post-natal anatomy of the venous system examined from the point of view of function, is expressed in the opinion of Claude Bernard: "*parmi les connaissances nombreuses que le physiologiste doit posséder l'anatomie se place sans contredit au premier rang.*" But the anatomists come in for a goodly share of reproof, deserved but kindly given. The anatomical literature on veins is still too much riddled with lacunae and errors, offers no adequate accounts of the venous system in the lower animals, pays little enough heed to age differences and individual variability, separates too rigidly structure and function and is prone to content itself with descriptions of cross- and longitudinal sections when the painstaking making of enlarged models is needed to give the physiologist a picture of the architecture of the vascular tube as a whole.

In the chapter entitled 'The Valves in Veins,' Franklin considers only certain aspects of these structures, since he had summarized the pertinent literature in 1927. With the help of the reviewer's figures, he delineates the embryology of the valves and then gives a concise and pithy account of their functional aspects, such as their occurrence in various veins, their distribution, mode of action, relative competence, etc. The reader regrets when the narrative ends, so attractively is it told.

The discussion in Chapter VI revolves around the problem of the amount of blood in circulation, as compared with that part of it which may be in reserve, either motionless or moving but slowly. The role of the spleen, liver, portal system, subpapillary venous plexus of the skin, lungs, uterine veins, and even of the larger systemic veins in serving as 'blood depots,' either dominantly or in emergency, is fully set forth.

The next chapter, referring to the comparative anatomy of the veins, is introduced with the startling assertion that "a collection of comparative anatomical facts is in itself of little value in the study of function," a statement which wins our approval when placed in proper perspective by a quotation from Charles Singer: "The mind is so constructed that it can take little interest in the accumulation of instances unless it can adduce general laws therefrom." Franklin maintains that "the only large part of the comparative anatomy of the venous system, in which there are suggestions of functional significance among the recorded facts, is that which is concerned with the vascular arrangements in diving birds and mammals." But he justifies the inclusion of this chapter on the grounds that "it may save others from a laborious search through a literature of a hundred and fifty years," and that "an experimental approach to the subject is in large measure impossible."

Chapter VIII deals in detail with the structure and behavior of the venules, as demonstrated by different investigators. The contractility of these amuscular channels, their role in diapedesis, in the production of tissue fluid, and in reabsorption, their part in the 'white' and 'red reactions' of the human skin, and the nature of their response to adrenalin, pituitary extracts, histamine, electrical stimulation, etc. are some of the manifestations considered.

Chapters X, XI and XII have as their caption: 'Veins and the Nervous System,' and are like most of the remaining chapters chiefly physiological in scope. Here, we are confronted with the most difficult part of the book to read and grasp. Its author cannot be held to account, for in its organization and arrangement his summary of the wealth of relevant literature is as good as the refractory subject permits; there are the different physiological aspects of the problems, the different methods of attack and their modifications, the different experimental animals used, the many different veins and related parts of the circulatory system tested, and the different and often conflicting observations made, all of which conspire to yield conclusions that are not easily formulated—least readily in a review.

The next seven chapters (XIII–XIX) treat of the factors which govern the venous return to the right and left sides of the heart in higher animals, and to the heart as a whole in lower animals.

The first of these chapters reviews the role of the heart, which is the main force, the *vis-a-tergo*, in the return. Then the effects of gravity and body posture on the circulation are discussed. Our interest is held throughout, from the first observations by Richard Lower in 1669 to the modern work after Leonard Hill on the physiological mechanisms involved. We are made clearly aware of the great difference of compensatory powers according to species, and of the human individual differences and variations in health and disease, how for example, in certain and frequently dramatic conditions—shock, fright, sea sickness, etc.—failure of compensation occurs, and how the body reacts to prevent such consequences; witness the soldier tightening his belt before going into action.

That muscular activity has a definite accelerator and augmentor effect upon the venous return, and thereby upon the circulation of the blood as a whole, is the thesis established in Chapter XV. Similarly, the attempt is made (Chapter XVI) to measure the effects of the contraction of involuntary muscle upon the venous return.

In Chapter XIX Franklin has achieved the task of providing us with a most lucid summary and critique of the different methods employed during the past two centuries to demonstrate the nature of the respiratory effects on the venous flow, and to measure them in different areas of the venous system (caval, portal, pulmonary, etc.).

Chapter XX, beginning with the work of the clergyman, Stephen Hales, the first one to measure venous pressure (1733), presents the technique of determining the fluctuating states of the 'postural activity' or 'tonus,' and of the pressure gradients within the vascular system, both in different species and also under varying conditions, and reveals the practical difficulties of actual recording.

The chapter on the movements of the blood in veins is one of the most interesting parts of Franklin's book. Since the growth of knowledge on this subject was and is dependent on the advances in technical equipment, the dictum '*Die Methode ist Alles*' is particularly apt here. We need merely to recall the gradual development and refinement of Ludwig's 'Stromuhr' (1867) and Cybulski's photo-haemotachometer to see the increasing precision in the measurements of the rate and amount of the blood flow. Another part of the chapter pertains to 'stream lines' in veins, that is, currents from confluent channels flowing along for long distances in a common stream bed without losing their individual characteristics. Though the results outlined by Franklin represent merely a beginning, "they make it obvious that the physiologist of the future will have to know the blood paths within the *venae cavae* and other vessels in the same way that he now knows the courses of nerve fibers within the central



nervous system." A third section of the chapter discusses arterio-venous anastomoses and their reactions, which especially since 1930 have received greater attention.

Chapter XXII, entitled 'Clinical,' brings the essential knowledge pertaining to intravenous injection, varicose veins, varicose ulcer, varicocele, and haemorrhoids, venous spasm, thrombosis, phlebitis, pathological arteriovenous communications, and mention of the other more miscellaneous conditions (thrombo-angeitis obliterans, phlebosclerosis, air emboli, etc.). The subject of intravenous injection is an especially interesting one because of the history of development of this therapeutic procedure from the times of Christopher Wren, Major, Caspar Scotus, and Elscholtz ('Clysmatica Nova,' 1665) in the middle of the 17th century. Even though Franklin characterizes his own summary of the knowledge on varicose veins as merely an introduction to the understanding of this most widespread disorder of the venous system, its excellence and thoroughness has given the fundamentals in the definition, frequency, etiology, heredity, different types, treatment, etc. of varicosis. To the anatomist the figure from W. Turner Warwick's book ('The rational treatment of varicose veins and varicoele,' '31) showing schematically the normal valvular mechanism of the lower limb, will have a special appeal.

A resumé of the application of various photographic techniques—stereophotography, ordinary cinematography, color cinematography, infra-red photography, microphotography, ordinary radiology, x-ray cinematography—constitutes chapter XXIII. The statement that "in physiology, photographic recording tends to reduce the number of animal experiments needed to ensure widespread acceptance of new discoveries" will hardly be denied.

The last chapter is prefaced with a quotation from an anonymous letter in 1890 addressed 'to Edinburgh Professors by a Medical Student' (now known as J. S. Haldane): "Physiology may be compared to a house partly built, partly building, and partly being rebuilt." This excerpt from a well-aimed criticism Franklin diverts to the defense of his own book, though the reader will attest that the merit of it is such, it demands no defense. He has tried "to show what is permanent in our knowledge of veins, to indicate where additions of value are even now being made to it, and where opinions about certain aspects of it are changing in the light of fresh research." But his attitude remains a tentative one, and is aptly expressed in the words of Sir James Frazier: "Now, as always, I hold all my theories very lightly, and am ever ready to modify or abandon them in the light of new evidence."

The bibliography contains over 1100 titles. It indicates the prodigious labor required to produce this most inclusive volume on the subject of veins. That he was able to achieve it in his spare time in the short span of 3 years is evidence of his unflagging interest, energy and industry.

Finally, it is a pleasure to point to the excellent craftsmanship which the publisher, Charles C. Thomas, has put into the printing of the book to make it consonant with the high quality of its content.

OTTO F. KAMPMEIER.

# 'OVOID BODIES' FROM THE OVIDUCTS OF RABBITS

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ONE PLATE (NINE FIGURES)

## I

In his recent review of the literature on the transport of ova and sperm through the oviducts, Hartman ('32, compare Allen, 'Sex and Internal Secretions') mentions that he occasionally found round masses of epithelial cells in the oviducts of the opossum, which had acquired an albuminous layer as well as egg envelopes. He also points out that these 'eggs without a vitellus' are comparable to similar structures found in the oviducts of chickens (Patterson, Pearl and Surface) consisting of bits of faeces or feathers which are surrounded by egg white, egg membrane and shell.

Such 'eggs without a vitellus' are often met with in the examination of the oviducts of rabbits, rats and mice (plate 1). These bodies generally resemble eggs so closely that on casual inspection they may be mistaken for true ova, which justifies the term 'eggs without a vitellus' or 'yolkless eggs' employed by Hartman. The term 'ovoid bodies' which has been chosen in the title of this paper is preferable, however, as it avoids any implications as to the origin of these bodies from the ovaries.

In the following experiments an attempt has been made to elucidate the origin and significance of the ovoid bodies in the oviducts of rabbits. It has been demonstrated, furthermore, that these structures could be utilized for physiological studies of the secretory activity of the oviducts.

## II

The interpretation of the origin of the ovoid bodies given by Hartman and other authors is without doubt the correct one; nevertheless it seemed desirable to find actual proof that these bodies are not derived from the ovary.

The oviducts and uteri of over 100 rabbits were flushed, and the eggs and any ovoid bodies present were recovered. The rabbits were killed at various time intervals after mating, ranging from 1 hour to 7 days. In all the cases where the work was done with some care it was found that the number of eggs recovered from the washing agreed with the number of ruptured follicles. There were no ruptured follicles to account for the origin of ovoid bodies from them. A number of mice in all stages of the oestrous cycle were dissected and any eggs that might be present in the generative tracts were recovered. In mice it is more difficult to obtain the full complement of eggs from one ovulation. Figures 1, 3 and 6 show ovoid bodies recovered from the oviducts of mice, and figures 4, 5 and 7 show ovoid bodies from rabbits. In the rabbit it was found that in all cases where the number of eggs recovered agreed with the number of bleeding points on the surface of the ovary, the ovoid bodies present were supernumerary. Histological sections always showed that atretic eggs degenerated within the ovary.

In mice the conditions seem to be different. Several cases were found where atretic follicles had ruptured. It is therefore possible that the ovoid bodies of this species are derived from the ovary. Furthermore mice do not coat their eggs with albumin, and ovoid bodies are present only after ovulation. The indirect evidence indicates strongly that in the mouse atretic eggs are ovulated occasionally.

If one accepts the theory that in the rabbit the ovoid bodies are formed by deposits of albumin, it should be possible to produce them artificially by introducing foreign particles of suitable size into the oviducts during the secretory period. A number of such experiments were performed in which foreign matter was introduced into the fimbriated end of the



oviducts. The impetus to these experiments came from accidental findings in connection with experiments to test the viability of rabbit eggs by transplanting them into suitable recipients. During such transplantations cellular debris was occasionally introduced into the oviducts of the recipients together with the eggs. Autopsies revealed that such debris had received an albumin coating the thickness of which depended to some extent on the length of the time interval between ovulation of the recipient and the operation. Bits of debris implanted into unmated rabbits had very little or no albuminous coating (fig. 2), while cellular debris implanted shortly after ovulation (fig. 8) was generously coated. Even spermatozoa or bacteria became clumped and encased in albumin. Figure 9 shows a stained section of three ovoid bodies which had formed around clumped bacteria. In order to have a conclusive test particles of carbon or stained fragments of cellular debris were introduced into the oviducts of several mated and unmated rabbits. If the autopsy was performed before the particles had had time to pass out of the fallopian tubes, it was possible to recover ovoid bodies in addition to the eggs in the mated rabbits; while in the unmated does the debris was found uncoated (figs. 2 and 8).

These experiments show conclusively that ovoid bodies may be obtained experimentally by introducing foreign particles of suitable size into the oviducts of rabbits after ovulation has taken place. No experiments were performed on mice.

There is one point which arises in connection with the experiments on ovoid bodies. It concerns the length of time elapsing between ovulation and arrival of the ova in the uteri and the mechanism which regulates the descent of the fertilized ova in the oviducts. It may be assumed that mechanical forces play an important role (peristalsis of the oviducts) and that the size of the eggs is a factor to be considered. Figure 7 shows a number of eggs in addition to an ovoid body. The latter is of considerable size. It is quite common to find ovoid bodies of all sizes ranging from less than one-tenth to about three times the diameter of normal eggs plus their albumin

coating. Since the eggs and the ovoid bodies pass into the uteri at about the same time it is obvious that the size of the eggs or of the ovoid bodies is not a very important factor in the regulation of the time interval required for the passage through the oviducts. Another startling phenomenon is the discrepancy in size between true eggs and ovoid bodies. Although the thickness of the albuminous layer around the egg varies from animal to animal and to a very slight extent even in the same animal, it does not exceed an upper limit which is in the rabbit about the diameter of the vitellus. Ovoid bodies, however, seem to grow to enormous proportions, often reaching three to four times the diameter of an uncoated egg. This suggests the possibility that there is an intrinsic mechanism which regulates the thickness of the albumin layer.

Another point of interest in connection with these experiments is the absence or feeble development of ovoid bodies in the oviducts of unmated rabbits. Histological studies of the epithelial lining of the oviducts during various stages of the sexual cycle and especially during the descent of the ova in the ducts throw some light on the problem. Such studies have been carried out by Katz ('11), Snyder ('23), Moreause ('13), Allen ('22) and other writers. According to Katz the epithelium of the fallopian tubes undergoes cyclic changes. The secretory phase coincides with early pregnancy, pseudopregnancy or prooestrus. Moreause ('13) found definite changes in the ciliated cells of the isthmus of the rabbit during ovulation, while according to Allen ('22), the secretory phase in the mouse involves some cells of the ampullae. Westman ('30) maintains that in rabbits the corpora lutea are responsible for the development of the eggs as well as for the secretory activity of the tubes. Westman's observations are supported by recent experiments performed in this laboratory (Burdick and Pincus, '35), which prove that injections of theelin cause retention of the ova within the oviducts and produce degeneration of fertilized ova. The theelin injections probably interfere with the normal functioning of the corpora lutea. The effect of theelin inhibition injection upon the secretory activity of the oviducts is being studied at present and it is hoped

that important information will be gained regarding the hormonal regulation of the secretory activity of the oviducts.

I should like to express my thanks to Mr. Burdick for the loan of a number of photomicrographs of ovoid bodies of mice and rabbits, some of which are included in plate 1.

#### SUMMARY

1. The 'ovoid bodies' found in the oviducts of rabbits after ovulation are formed from cellular debris which acquires a coat of albumin. In mice, however, they are probably derived from atretic follicles.

2. It is possible to induce the formation of ovoid bodies in rabbits artificially by introducing foreign particles into the oviducts.

3. The formation of ovoid bodies after injection of debris may furnish a suitable method of studying the hormonal relations of the secretory activity of the oviducts by physiological means.

4. Observations on the difference in thickness of the albuminous layer around ovoid bodies and eggs seem to indicate that there is some mechanism for regulating the amount of albumin which is deposited on each egg.

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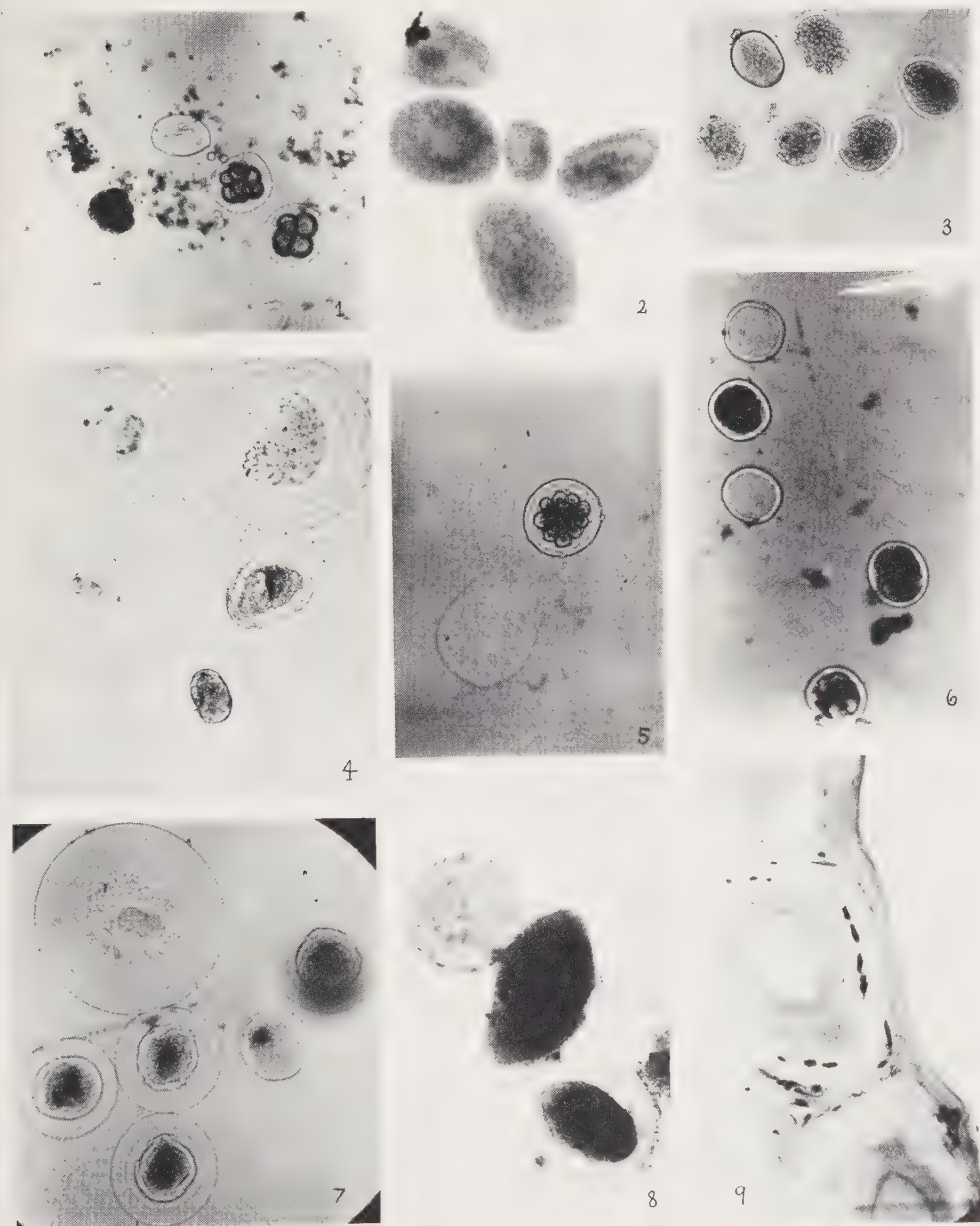
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## PLATE 1

### EXPLANATION OF FIGURES

- 1 Three mouse eggs in the early morula stage and an ovoid body (at the top).
- 2 Cellular debris surrounded by a thin layer of albumin, from the oviduct of an unmated rabbit.
- 3 Six degenerating mouse eggs and on the bottom an ovoid body.
- 4 Rabbit eggs and ovoid bodies of various sizes.
- 5 Rabbit egg in morula stage fused with an ovoid body.
- 6 Three mouse eggs and two ovoid bodies.
- 7 Five degenerating rabbit eggs which were retained in the oviducts following theelin injection and an ovoid body of large size.
- 8 Cellular debris stained with eosin and injected into the oviducts where it became coated with albumin. On top an ovoid body formed around unstained material.
- 9 Stained sections of three ovoid bodies formed around clumped bacteria, which had been introduced into the oviducts.







# A DIFFERENTIAL COUNTERSTAIN FOR TONED SECTIONS OF PYRIDINE SILVER PREPARATIONS OF PERIPHERAL NERVES

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ONE FIGURE

## INTRODUCTION

The development of the pyridine silver technique by Ranson gave an impetus to the study of types of nerve fibers in peripheral nerves, particularly the unmyelinated fibers. Previous to that time axon studies were largely confined to osmic acid preparations in which only the myelinated fibers were stained. Sections of nerves stained by the Ranson method show a definite contrast between the myelinated and the unmyelinated fibers and for qualitative purposes the unmodified technique is unsurpassed. However, as recently pointed out by Jones ('36), many preparations which would be unsatisfactory for quantitative purposes may be successfully utilized if they are toned with gold chloride, but toning has the disadvantage of destroying the contrast which previously existed between the myelinated and unmyelinated fibers and except for differences in size all fibers look alike. Therefore, as emphasized elsewhere (Foley, '36), counterstains are indicated if one wishes to differentiate the two types of axons. Although counterstaining of toned sections of nervous tissue is not new, the use of counterstains as aids in quantitative studies of peripheral nerves is recent (Foley and DuBois, '37). For a while, as pointed out in a previous publication (Foley, loc. cit.), the formula of Heidenhain (Romeis, '28, the so-called azan technique), seemed to hold most promise

of fulfilling the requirements of a differential stain, for, when it was applied to sections of peripheral nerves which had been silvered with the Davenport technique, the nuclei of the Schwannian syncytium and the stroma stained bright red, the remnants of the myelin sheaths orange and the collagenous tissue blue or green. However, the writer has not been able to obtain this contrast with toned sections of pyridine silver material as the red azocarmine of the Heidenhain combination stains both the nuclei and the remains of the myelin sheaths with equal intensity. Attempts to surmount this difficulty have resulted in the development of a counterstain which seems to fulfill the essential requirements of a differential stain for toned sections of pyridine silver material. In the technique the nuclei are stained with Fuelgen's method and the other non-nervous tissue elements with Heidenhain's acidified aniline blue-orange G mixture, in which light green or fast green<sup>1</sup> is substituted for aniline blue. Toned sections of pyridine silver material which are counterstained by this technique show the axis cylinders black or steel gray, sheath and fibroblast nuclei red, myelin sheaths yellow and connective tissue green. The remnants of the myelin sheaths stain so distinctly in favorable preparations that quite accurate counts can be made of these structures in counterstained preparations, and in such instances counterstaining obviates the necessity of securing special blocks for osmic acid staining.

#### PREPARATION AND FIXATION

Staining with silver closely follows the procedure outlined by Ranson in his most recent variation of the pyridine silver technique (Ranson and Davenport, '31). However, the method of preparing the tissue for fixation and staining with silver is slightly different and, for purposes of completeness, the steps are listed. The animal is killed with some suitable

<sup>1</sup> The author is indebted to Dr. S. I. Kornhauser for suggesting the use of fast green. It is supposed to be more stable than light green and gives a darker coloration. The writer believes that the substitution of a green stain, light or fast green, for the aniline blue in Heidenhain's formula is of value since the green dye makes the gray or black axons stand out more sharply.

anesthetic and perfused with normal saline until the salt solution runs clear. The nerve is then cleared of surrounding structures and areolar tissue, and blocks of tissue are isolated by tying them off with black (proximal) and white (distal) silk threads; after which, the segments of nerve are removed (by cutting between the threads with a pair of sharp scissors) and put under slight tension as described under figure 1. Following stretching, the tissue is fixed for 6 to 8 hours in ammoniated ethyl alcohol (99 cc. absolute ethyl alcohol, 1 cc.

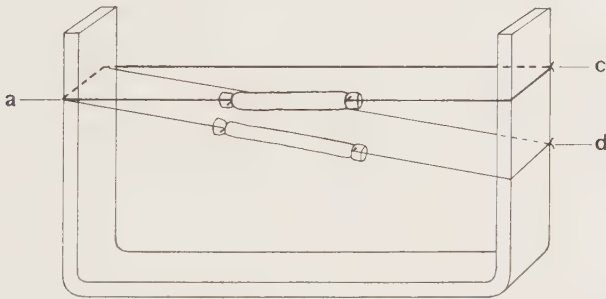


Fig. 1 Glass U's for stretching nerves are made from flat glass strips secured by cutting them from waste lantern slide covers or old lantern slide plates. The strips measure approximately 0.5 cm. in width and 9 cm. in length. The cut surfaces are polished with a file and the strip is bent, after heating, into the form of a U. The arms should be at least 2 cm. in length to give sufficient stretching distance. The nerve is tied taut, close to the extremities of the arms (a, c); then, one end (knot end) of the string is shifted into position (a, d) until the desired tension is secured. This simple piece of apparatus utilizes the same principle as that formerly used in stretching on a solid glass slide but has the advantage over the latter in that the glass U permits prompt and immediate fixation on all surfaces the minute it is dropped into a vial of fixative. Vials measuring 24 mm. by 50 mm. and 24 mm. by 80 mm. serve all purposes and readily accommodate glass U's with a 2 cm. arm length.

The author wishes to thank Dr. Eleanor A. Hunt for making the drawing for the above figure.

of 28% ammonium hydroxide). At the end of the period of preliminary fixation the tissue is removed from the stretchers and the knot and the portion of the nerve it embraces is cut from the end of the nerve from which sections are desired. The nerve is then threaded through a piece of spinal cord or cortex which was removed and stored in the ice box



when the animal was terminated. The nerve is pulled through the coating substance with a fine needle in such a way that it is covered superficially with a thin layer of white substance and pia mater. The coated nerve is now returned to the fixative for the remainder of the initial 24-hour period; at the end of which time the tissue is removed, trimmed with a sharp razor blade to a diameter of about 2 mm. and returned to fresh fixative for an additional 24 hours.

*Treatment after fixation.* After fixation the tissue is rinsed in distilled water and put into Merck's reagent grade pyridine for 24 hours. Following the pyridine treatment the nerves are washed for 36 hours in many changes of distilled water, the water being changed every hour during the daytime.

*Silvering.* After washing, the tissues are silvered in a 2% aqueous solution of silver nitrate for 5 days, changing the silver on the third day. This practice is in agreement with the latest recommendation of Ranson (Ranson, Foley and Alpert, '33), who finds that the lengthening of the period of silvering is helpful in securing a clearcut staining of the smallest fibers.

*Reduction.* At the end of 5 days the silver is poured off and the tissue is briefly washed in distilled water and put into Ranson's pyro-formalin reducer (Merck's reagent grade pyrogalllic acid, 4 gm.; Merck's reagent grade formaldehyde, 5 cc.; distilled water, 95 cc.) for 48 hours.

*Dehydration and embedding.* After reduction the tissue is passed through grades of alcohol to absolute by increments of 10%, remaining in each percentage grade for a minimum of 30 minutes.<sup>2</sup> After a period (12 to 15 hours) in absolute alcohol the tissue is placed in ether alcohol for 8 to 12 hours, after which it is put in thin celloidin (3%) for 2 days and

<sup>2</sup> Following the practice of Jones ('36), the coating of cord or cortex is not removed from the nerve during dehydration nor at any succeeding step. It acts as a buffer against distortion currents during the dehydration process and protects the nerve fibers from any slow fading action of the dehydrating and clearing fluids. Likewise, since it in itself is of no direct value, it can be pulled upon in spreading sections.

thick celloidin (6%) for 5 days. Concentrations of less than 3% should be avoided since dilute solutions of nitrocellulose tend to fade silver preparations. At the end of the period of infiltration the nerve is taken from the thick celloidin, rinsed in thin celloidin, and then rapidly rolled on filter paper to remove all surface celloidin since adhering nitrocellulose is detrimental to successful embedding and subsequent spreading of cut sections. If, after the rolling process, the surface of the coated nerve has a smooth and glistening instead of a dull and rough appearance, the periphery should be lightly scraped. After the surface of the nerve has been suitably prepared, it is placed in a mixture of equal parts of normal butyl alcohol and benzene in which it is allowed to remain, with occasional shaking, for 2 hours. The butyl-benzene mixture is poured off and the tissue is transferred to pure benzene for 2 hours, during which time four changes of benzene are made. The nerves are removed from the benzene at the completion of the clearing process and placed in the paraffin oven (45°C.) in a mixture of equal parts of paraffin (56° to 58°C.) and benzene for 24 hours, following which they are placed in pure paraffin overnight (approximately 16 hours). The following morning they are put in a fresh change of paraffin for 8 hours, after which they are embedded.<sup>3</sup>

<sup>3</sup> The double embedding technique is superior to the usual paraffin methods since it imparts a greater sharpness and brilliancy to the collagenous tissue and the remnants of the myelin sheaths and as emphasized by Jones ('36), double embedding effects better resolution of the fine fibers. In the hands of the author the particular method herein described has proved superior to other double embedding techniques in that serial sections can be readily secured and later spread on albumenized water with practically the same ease as if the tissue was embedded in paraffin alone. However, for the benefit of those who may be inexperienced in handling double embedded material, the following suggestions are offered whereby smooth sections may be secured: 1) use only five or six sections to a row; 2) place the ribbon on cold albumenized water and separate the wrinkles by gently pulling on the coating substance around the nerve; 3) put the slides on a warming plate raised to a temperature just sufficient to render the paraffin pullable and stretch the ribbon until the sections appear smooth—a gentle pull on the coating substance surrounding the nerve is often of value as the sections start to move; 4) if wrinkles still persist, smooth sections may usually be secured if the paraffin is broken on either side of the section before steps one to three are carried out.

*Counterstaining.* Sections of the silvered nerve are cut and spread in the usual manner, the paraffin is removed with xylene and the slides are passed from absolute alcohol through decreasing grades of alcohol to water. From water the sections are toned with a 0.2% aqueous solution of gold chloride to which 10 drops of glacial acetic acid (Jones, '36) has been added. As previously pointed out (Foley and DuBois, '37), the toning process must be carefully watched under the microscope. After toning the slides are rinsed very quickly in distilled water and immersed in 5% aqueous solution of sodium thiosulphate for 5 minutes, after which they are thoroughly washed in running tap water and rinsed in several changes of distilled water. Following rinsing in distilled water, the sections on the slide are treated with Fuelgen's technique<sup>4</sup> (McClung, '37) to stain the nuclei. After nuclear staining the slides are taken from distilled water (step three of footnote 4) and put into a 5% aqueous solution of phosphotungstic acid for 15 to 60 minutes; after which, they are placed in a dilution (stock solution, 20 cc.; distilled water, 30 cc.) of the following stain mixture for 1 to 5 hours.

Fast green, F.C.F., National Aniline Co. (94%, representing 0.470 gm. actual dye), 0.5 gm. or light green, S.F., Coleman and Bell Co., (70%, representing 0.35 gm. actual dye), 0.5 gm.; orange G, Coleman and Bell Co. (90%, representing 1.8 gm. actual dye), 2.0 gm.; glacial acetic acid, Merck's reagent grade, 8 cc.; distilled water, 92 cc.

At the termination of the staining interval (determined by trial) in fast green-orange G or in light green-orange G, the sheaths of the axons and the collagenous tissue in the sections are differentiated under the microscope by thoroughly rinsing the slides in at least two changes of 95% alcohol. In order

<sup>4</sup> Since the method is here adapted to tissue for which it was not developed, it is necessary to list certain variations which are essential for successful nuclear staining; namely, 1) hydrolysis in hot hydrochloric acid should be maintained for 15 to 30 minutes; 2) the staining time in fuchsin-sulphurous acid is lengthened to a minimum of 1 hour or a maximum of 4 hours (2 hours gives excellent results); and 3) after staining and rinsing in the sulphurous acid baths the sectioned tissues should be thoroughly washed in distilled water to remove the last trace of free sulphurous acid.

to secure critical differentiation it is necessary sometimes to pass the slides back and forth from 95% to 75% alcohol. After differentiation is completed, the slides are passed through two changes of absolute alcohol, three changes of xylene, and the sections are mounted in dammar.

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# THE NERVE SUPPLY OF THE HYPOPHYSIS OF THE CAT<sup>1</sup>

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EIGHT FIGURES

The innervation of the different lobes of the hypophysis cerebri has received rather sporadic attention since Bourguery (1845) first observed passing to it fibers which were believed to be of sympathetic origin. Most later investigations have been limited either to a study of the extra-glandular course of the fibers, or to studies dealing solely with their morphology within the various hypophyseal lobes. In view of the scattered nature of our information on this important matter it was considered worth while to make it the subject of a more complete study in a single animal species.

## HISTORICAL

*Anterior lobe.* Berkley (1894), using chrome silver methods, furnished descriptions of nerve fibers in the pituitary which he believed to be of sympathetic origin. The finer ramifications of these fibers were described as terminating between the epithelial cells.

Dandy ('13) has made a very excellent study of the external connections of the hypophyseal sympathetic fibers in the dog and cat. His paper furnishes very clear-cut illustrations of the origin of fibers from the carotid plexus on the circle of Willis, and supports his contention that the key to the nerve supply of the pituitary lies in its arterial connections. By use of methylene blue technique he was able to

<sup>1</sup>A portion of a dissertation submitted in partial fulfillment of the requirements for the degree of doctor of philosophy in the University of Buffalo.

demonstrate sympathetic filaments which pass along the blood vessels to the stalk of the hypophysis and penetrate into the substance of the anterior lobe. He observed that the majority of the arterial branches carried only one or two small filaments. The fibers were described as being unbranched and having a rather direct course to the glandular hypophysis.

A more extensive description of the fibers in the anterior lobe has been offered recently by Pines ('25). Using methylene blue as well as silver methods he demonstrated that the anterior lobe is richly supplied by amyelinic nerve fibers of sympathetic origin. He describes a very intimate relationship between fibers and gland cells, and pictures fibers that form intercellular nets with coarse varicose thickenings. From these varicosities arise other fibrils which, after branching and reanastomosing, terminate as end-nets which closely invest the gland cells. The small fibers of the end-nets are stated to possess fine node-like thickenings which lie against the free surface of the cells. This investigator subscribes to the belief that the fibers are secretory in function.

Croll ('28) using modifications of Ranson's pyridine silver technique was able to demonstrate a few fibers in the anterior lobe. These unmyelinated fibers were seen to run between the cells of the anterior lobe where they occasionally terminate in knob-like endings. She emphasizes that poor penetration and the capricious action of the silver probably account for her inability to demonstrate nerves more consistently in all parts of the glandular hypophysis. She is inclined not to regard these fibers as vasomotor, but as secretory in function.

*Posterior lobe.* Cajal (1894) working with human material, gave rather conclusive evidence for the presence of nerve fibers in the posterior lobe, and in so doing supplied much information in favor of the nervous character of the infundibular process—a controversial point at that time. Although he did not definitely locate the nucleus of origin, he described a well-developed bundle of fibers which, arising in the brain, passed down the stalk to terminate as a rich plexus

in the posterior lobe. The fact that the pars nervosa is largely nervous in nature has sometimes been lost sight of, as is attested by Cowdry's ('22) statement that nerve fibers are of rare occurrence in the pars nervosa.

Pines ('25) confirmed the observations of Cajal and determined that some of the fibers arise from nuclei bilaterally placed in the floor of the third ventricle, somewhat above and behind the optic chiasma. This nucleus of origin he termed the hypophyseal nucleus. Here fibers arise from uni-, bi-, and sometimes tripolar cells, converge medioventrally down the infundibular stalk into the infundibular process where they terminate as a rich plexus.

Greving ('26) traced fibers from the nucleus supraopticus (the nucleus hypophyseus of Pines) and designated the fiber pathway from this center to the pars nervosa as the tractus supraopticus-hypophyseus. Another group of cell bodies, the nucleus paraventricularis in the wall of the third ventricle, was believed by him to be associated with this tract. He described fibers which arise from this nucleus and pass downward as the tractus paraventricularis-cinereus to join either the nucleus or tractus supraopticus-hypophyseus.

Evidence in favor of the nucleus supraopticus as the point of origin of the tractus supraopticus-hypophyseus has been supplied by Kary ('24) and Lewy ('24). These workers observed regressive changes in the cells of the supraoptic nuclei in cases involving lesions of the infundibular stalk and process. More recently workers of the Ranson school ('35-'37) have produced experimental lesions of the hypothalamus in adult cats, the lesions being so placed as to interrupt the supraoptico-hypophyseal tract. The animals, besides showing a disturbance of water balance, showed degeneration of unmyelinated fibers to the posterior lobe, as well as degeneration of cells in the supraoptic nuclei.

The manner of termination of nerve fibers in the posterior lobe is not well understood. Cajal ('11) has described fibers from the supraoptico-hypophyseal tract which form a rich plexus between the epithelial cells of the pars intermedia.

Tello ('12) confirmed this observation and also demonstrated nerve fiber terminations on the vessels. Here the fibers penetrate the perivascular connective tissue and end with small granular enlargements. Greving ('25) described no definite fiber terminations, but was able to follow for considerable distances, fibers having varicose swellings and surrounded by oval cells of the endoneurium. Pines ('25) also traced many of the fibers into the pars intermedia.

In the pars nervosa of the rabbit hypophysis Croll ('28) identified nerve terminations in the walls of blood vessels which resemble sympathetic endings. She also observed thin fibers extending around the blood vessels which she believed to be too numerous to be connected only with the vessels. Similar fibers were also seen in the pars intermedia.

Ibanez ('34) described fibers which terminate in a complicated fashion in cell masses of the neural lobe and others, with enlargements on their extremities, which end in the zone between pars intermedia and pars nervosa. He also described fibers which terminate between the epithelial cells of the pars intermedia.

Bucy ('30) has supplied an account of the manner in which the fibers of the supraoptico-hypophyseal tract terminate in the pars nervosa in cattle. These fibers are described as 1) terminating as large end bulbs; 2) dividing into two or three end branches which appear to surround a pituicyte; and 3) ending blindly in the connective tissue of the capsule.

Dandy ('13) reports that only a few fibers of sympathetic origin accompany the posterior hypophyseal artery to the posterior lobe.

The writer is greatly indebted to Dr. Wayne J. Atwell, under whose direction this work was carried out.

#### MATERIAL AND METHODS

This study is based entirely on the cat; thirty-three animals were used. Under urethane or ether anesthesia the carotid arteries were cannulated and the vessels washed out with



about 100 cc. of isotonic saline solution. From 150 to 200 cc. of fixing fluid was then perfused through the head. The animal was decapitated, the calvarium removed, and the entire head then placed in a quantity of fixing fluid for about 2 hours. The pituitary and a considerable amount of the attached brain stem were then dissected free and the fixation completed.

Eighteen of the glands were impregnated according to Ranson's pyridine silver technique. Four preparations were made following various modifications of the Bielschowsky method for the demonstration of peripheral nerves. The method outlined by Bodian ('36) for staining mounted paraffin sections was found to be advantageous and was used in six animals.

The impregnated specimens were embedded in paraffin, sectioned at 12  $\mu$  and mounted serially.

Five animals were sacrificed in attempting supravital methylene blue methods, but although preparations suitable for tracing fibers outside the gland were obtained, the technique—in the hands of the writer at least—did not prove to be of value in tracing fibers within the glandular parenchyma.

In addition to the above material a small series of cats was used in an attempt to determine the origin of the nerve fibers passing from the carotid plexus to the hypophysis. These animals were subjected to bilateral removal of the superior cervical ganglion and cervical sympathetic trunk. The fascial investment of the common carotid arteries was also stripped off for a distance of about 1 cm. in order to obviate the possibility that fibers arising from the inferior cervical ganglion might reach the cranial cavity after passing to the carotid artery at a lower point in the neck. The cats were allowed to live for 30 days after the operation, a period of time which was considered sufficient to allow complete degeneration of nerve fibers having their cell bodies in the superior cervical ganglion. The four animals that survived for the required 30-day period were sacrificed in the usual way and the hypophysis prepared according to Ranson's pyridine



silver and Bodian's silver techniques. After perfusion, the nodose ganglion and surrounding connective tissue in the area previously occupied by the superior cervical ganglion were removed and sectioned serially in order to determine whether any remnants of the ganglion remained. The internal carotid artery and the proximal part of the external carotid were also removed and fixed so that a check might be made of the possible existence of accessory ganglia which have occasionally been reported in the human. Since the stellate ganglion was not removed, there remained the remote possibility that fibers might reach the hypophyseal region by way of the vertebral plexus. It is known that the majority of the fibers of the vertebral plexus do not reach the cranial cavity, but are given off as communicating rami to cervical nerves. However, to obtain an estimate of the number of fibers reaching the head by way of the vertebral plexus, the vertebral and basilar arteries and surrounding brain tissue were also removed, fixed and sectioned.

#### OBSERVATIONS

*Fibers from the carotid plexus.* As reported by Dandy ('13) the extensive plexus of unmyelinated fibers which is associated with the internal carotid arteries and circle of Willis gives origin to rather prominent bundles of fibers which, retaining their intimate vascular associations, accompany some of the superior hypophyseal arteries to the anterior lobe of the hypophysis. The number of fibers along a vessel is inconstant, but appears to be proportional to the size of the vessel accompanied (fig. 5). The course taken by the fibers in passing to the anterior lobe of the hypophysis varies in two particulars—a feature which appears to be dependent on the manner of vascularization of the anterior lobe. It has been demonstrated by the writer ('37) that blood reaches the anterior lobe by two distinct vascular pathways: 1) Arteries which arise from the Willisian circle and take an uninterrupted course to the anterior lobe, and 2) those which pass by way of the pars tuberalis where they form an extensive vascular plexus (fig. 4). (Wislocki and King ('36)

have made a previous demonstration of this feature of the circulation in the monkey. More recently Wislocki ('37) has made an extensive study of the hypophyseal blood supply in the cat.) Vessels comprising both of these arterial pathways are accompanied by nerve fibers arising from the carotid plexus. The bundles along the more direct vascular channels contain the most fibers, there frequently being seven or eight unmyelinated fibers on the wall of a vessel. A few of the numerous small arteries which penetrate the pars tuberalis have one or two fibers accompanying them.

The course taken by the fibers within the anterior lobe is extremely tortuous, so much so, in fact, that only short segments of them can be traced in a single section (fig. 2). They often accompany vascular channels for considerable distances, giving off frequent branches which pass between the epithelial cells. Occasional knob-like enlargements occur along the fibers and in favorable preparations club-like terminal pieces can be seen. These terminal parts frequently appear to have a slightly lessened affinity for silver, since the blackened fibers may be seen to give way to more lightly stained terminal enlargements which lie against the epithelial cells of the gland. This difference in staining intensity undoubtedly accounts for the low percentage of end bulbs seen in the preparations, for in many cases the terminal parts are not impregnated. The end nets described by Pines as closely investing the gland cells were in no case seen. Although it must not be assumed that the inability to demonstrate such nets in my preparations is construed as positive evidence against their presence, it should be mentioned that Pines' descriptions of the pericellular nets are very suggestive that he has confused nerve fibers with cellular investments of reticular connective tissue. This is particularly indicated by the fact that he used Bielschowsky methods which even in the best of preparations give some connective tissue impregnation particularly around the periphery of the gland where most of his pericellular nets were seen.

The nerve fibers which arise from the carotid plexus and pass through the pars tuberalis also show occasional enlargements along their course which bear relation to the epithelial cells of this part. These endings, diagrammatically indicated in figure 1, are similar to those described for the anterior lobe (fig. 2).

It has been generally assumed by previous workers that the fibers arising from the carotid plexus and passing to the



Fig.1 Diagrammatic representation of the innervation of the hypophysis indicating the origin and distribution of the nerve fibers. A., nerve fibers from the carotid plexus which pass to the anterior lobe, posterior lobe and pars tuberalis; B., fibers of the supraoptico-hypophyseal tract which are distributed mainly to the pars nervosa and pars intermedia, but a few of these fibers pass to the pars tuberalis and anterior lobe; C., fibers of the tubero-hypophyseal tract to the posterior lobe; O.C., optic chiasma.

anterior lobe of the hypophysis are entirely sympathetic in origin. An examination of the glands from animals which had suffered bilateral removal of the superior cervical ganglion 30 days before death proves that this is not the case. The glands of animals which survived for the 30-day post-operative period show approximately as many fibers as could be demonstrated in unoperated animals. Obviously quantitative estimates cannot be made nor are they particularly desirable. Technical difficulties with silver methods

make it impossible to obtain comparative quantitative estimates of fibers in normal and operated animals. However, the pertinent facts are that the vessels comprising the circle of Willis, its branches leading to the hypophysis, and the gland itself all possessed normal unmyelinated fibers following the operation. Serial sections of the carotid vessels yielded no evidence of accessory ganglia or isolated cell bodies that might be the source of these fibers. The vertebral arteries at their point of entry into the foramen magnum possessed a few fibers, but a consideration of their number and apparent distribution makes it doubtful that these fibers reach the hypophyseal vessels. It seems certain therefore, that the carotid plexus and the glandular hypophysis receive fibers which are not of cervical sympathetic origin.

*Hypothalamic fibers.* The nuclei of origin of the fibers of the supraoptico-hypophyseal tract seem to be fairly well established for the cat, and no attempt has been made to investigate this point further.

The abundance of neuroglia fibers in the pars nervosa and the impossibility of obtaining an impregnation of nerve fibers without some neuroglia taking the stain make it difficult with present methods to determine definitely the manner in which fibers terminate in this lobe. Some of them form a rich plexus which is intimately associated with the abundant vascular channels of the pars nervosa, while others terminate without specialized endings in regions occupied by numbers of pituicytes. The bulb-like endings described by Tello ('12) in the human neural lobe and by Bucy ('30) in cattle are also present in the cat. These arise from small tortuous fibers of the supra-optico-hypophyseal tract and the terminal end bulbs have well-formed protoplasmic sheaths. The fiber within the bulb is sometimes extensively coiled. These end pieces are variable in size, varying from 3 to 10  $\mu$  (fig. 8).

In the intermediate zone between pars nervosa and pars intermedia many of the fibers show terminal enlargements similar to those of the sympathetic fibers in the anterior lobe.



The pars intermedia is supplied by an abundance of fibers which have their origin in the supraoptico-hypophyseal tract. These fibers course inferiorly and give off numerous branches which take a tortuous course between the epithelial cells. A prominent fiber group is seen next to the residual lumen in figure 7. The terminal end pieces are knob-like or club-shaped. Some terminate in such intimate relation with vessel walls as to suggest a vasomotor function, while others are in contact with the epithelial cells (fig. 3).

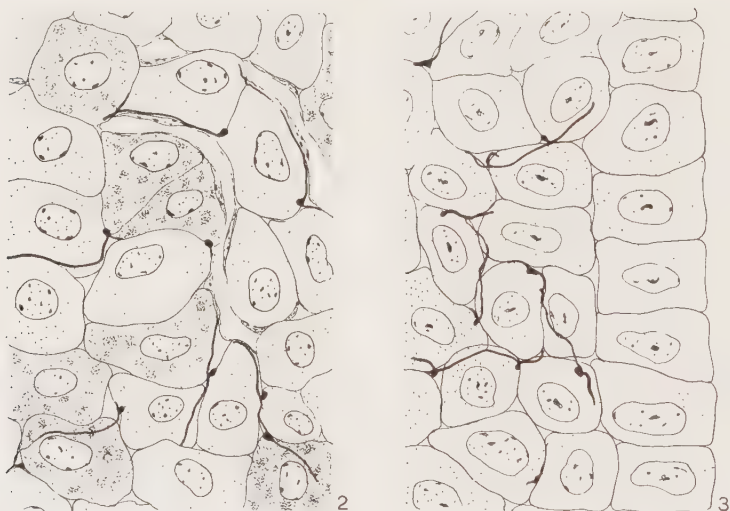


Fig. 2 Diagrammatic representation of nerve fibers in the anterior lobe showing the course of the fibers and the relation of the knob-like enlargements to the epithelial cells.

Fig. 3 Diagrammatic representation of the nerve fibers in the pars intermedia.

Cameron ('29) in referring to the distribution of the fibers of the supraoptico-hypophyseal tract has made casual mention of fibers which leave the infundibular stalk and terminate in relation to the epithelial cords of the pars tuberalis. This observation may be extremely important in the light of the present trend of experimental work designed to determine the relationship between pituitary and hypothalamus. Before Cameron's statement had come to my attention I had observed that there are a number of very small nerve fibers



which make their way through the peripheral zone of the infundibular stalk and enter the pars tuberalis (figs. 1 and 6). These fibers appear to take origin from the supraoptico-hypophyseal tract and leave the stalk either singly or in pairs. They frequently accompany vascular channels, but this is not without exception. The course of the fibers once they gain the pars tuberalis is, because of their tortuous nature, difficult to determine. Many of those which can be definitely traced become associated with fibers from the carotid plexus which have been described as accompanying the vessels in the pars tuberalis. The point of emergence of the fibers from the infundibular stalk is not constant enough to allow a definite localization. They may take origin close to the superior end of the pars tuberalis or more inferiorly along the infundibular stalk. The majority, however, appear to arise in the lower part of the stalk, i.e., in that region where the tuberalis vessels are entering the anterior lobe.

It also has been possible in some instances to trace a few unmyelinated fibers from the supraoptico-hypophyseal tract directly to the anterior lobe. These fibers penetrate the stalk below the point of juncture of pars tuberalis and anterior lobe, and can be traced for a considerable distance into the substance of the glandular lobe where they ramify extensively among the epithelial cells (fig. 2).

#### DISCUSSION

*Anterior lobe innervation.* In spite of the long accepted evidence provided by morphological studies that there exist in the anterior pituitary nerve fibers of an autonomic nature, the functional significance of such fibers still remain obscure. The theory that there is in operation a reciprocal endocrine activation and inhibition between the hypophysis and ovary obviated the need of a nervous pathway to explain phasic activation of the pituitary as regards its gonadotropic hormone. Recently, however, the failure to establish the actual existence of a pituitary inhibiting activity by estrogenic or corpus luteum hormones has cast doubt on the truth of the 'push-pull' theory as an explanation of cyclic ovarian activity.

Likewise, the experiments of Schweizer, Charipper and Haterius ('37) showing the non-cyclic behavior of the ovary after homoplastic anterior pituitary grafts in the guinea pig have suggested that ovarian rhythmicity may be traced to hypophyseal neural connections rather than to cyclic pituitary activation and inhibition through the mediation of ovarian hormones.

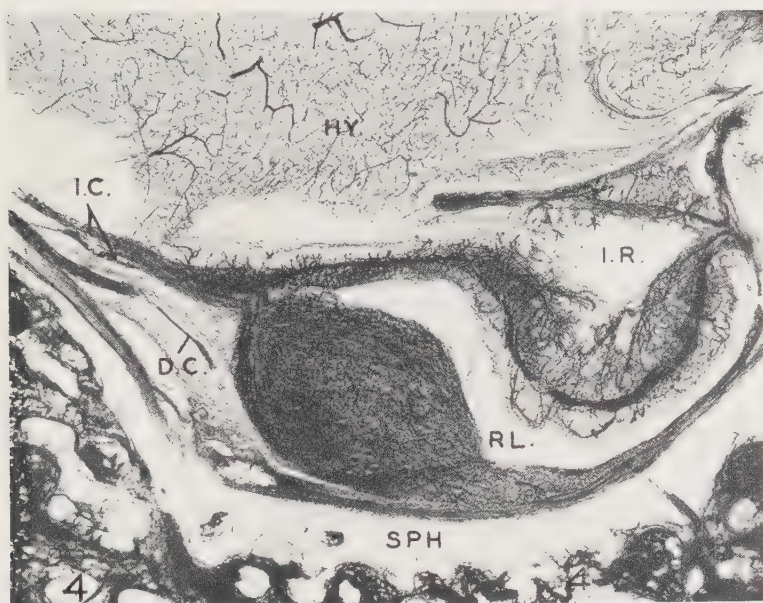
On the other hand it has long been believed that in certain animals there exists a nervous pathway between the central nervous system and anterior pituitary which functions as the efferent limb of a reflex arc involving impulses set up in the accessory sex organs. Thus in many amphibians ovulation is believed to be mediated by a hyperactive pituitary which has been stimulated to increased secretory activity through a nervous reflex initiated by the sexual embrace. Well recognized also is the peculiarity of the ovulatory phenomenon in the rabbit, which is not spontaneous as in most mammals, but is dependent for its initiation on the act of coitus. It has been shown that the actual discharge of the ovum takes place 10 to 12 hours after mating. This also is approximately the length of time required for artificial induction of ovulation by injection of anterior pituitary hormones. Moreover, if the animal is hypophysectomized within 1 to 1½ hours after mating, ovulation does not occur (Fee and Parks, '29).

Cushing ('32) recognized that a nervous mechanism must be involved, in this ovulatory response to copulation. He suggested that the nervous impulses initiated by the copulatory act must be directed to the hypophysis through the mediation of the inter-brain, a part common to all mating

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Fig. 4 Photograph of an injected cat hypophysis showing the arteries of supply to the anterior lobe. D.C., vessels of the direct arterial channel to the anterior lobe; I.C., vessels of the indirect arterial route passing by way of the pars tuberalis; R. L., residual lumen; I.R., infundibular recess; H.Y., hypothalamus; S.P.H., sphenoid bone.  $\times 24$ .

Fig. 5 Photograph showing the juncture of anterior lobe (A.L.) and pars tuberalis (P.T.). Entering the anterior lobe is a prominent artery (A.) accompanied by a group of unmyelinated nerve fibers (N.F.) which took origin from the carotid plexus. Pyridine silver.  $\times 260$ .



species. These impulses, he suggested, might be transmitted to the gland directly, or, as a more likely possibility, they might reach it in a more indirect route by way of the cervical sympathetics.

Marshall and Verney ('36) recently have shown that ovulation can be produced in the rabbit by electrical stimulation irrespective of whether the stimulus is applied through the brain or the lumbosacral cord. The observation is additional evidence in favor of a neuro-hypophyseal connection. That the nervous pathway is not by way of the cervical sympathetics as Cushing has suggested is indicated by the work of Hinsey and Markee ('33) who showed that rabbits in which the cervical sympathetic trunks had been bilaterally sectioned, when allowed to mate, gave birth to normal sized litters. Likewise, Haterius ('34) failed to induce ovulation in the rabbit by stimulation of the cervical sympathetic trunk. He claimed thus to have demonstrated that the nervous pathway, if one exists, is not through the cervical sympathetics.

Interesting in this regard are the observations of Chorobski and Penfield ('32) who, in investigating the question of vasodilator fibers in the meningeal vessels, demonstrated that a vasomotor mechanism exists in these vessels which is antagonistic to the sympathetic vasoconstriction and is therefore parasympathetic in nature. Anatomically they showed that there exists a small bundle of fibers which is recognizable as a distinct portion of the nervus intermedius of Wrisberg just proximal to the geniculate ganglion. These fibers pass uninterrupted through the geniculate ganglion to the greater superficial petrosal nerve. The investigators gave evidence that the bundle was synaptically interrupted in a small ganglion on the carotid artery from which post-ganglionic fibers continued on into the carotid plexus. Complete removal of all sympathetic nerve fibers entering the cranial cavity was shown not to reduce appreciably the number of normal intracranial perivascular nerve fibers.



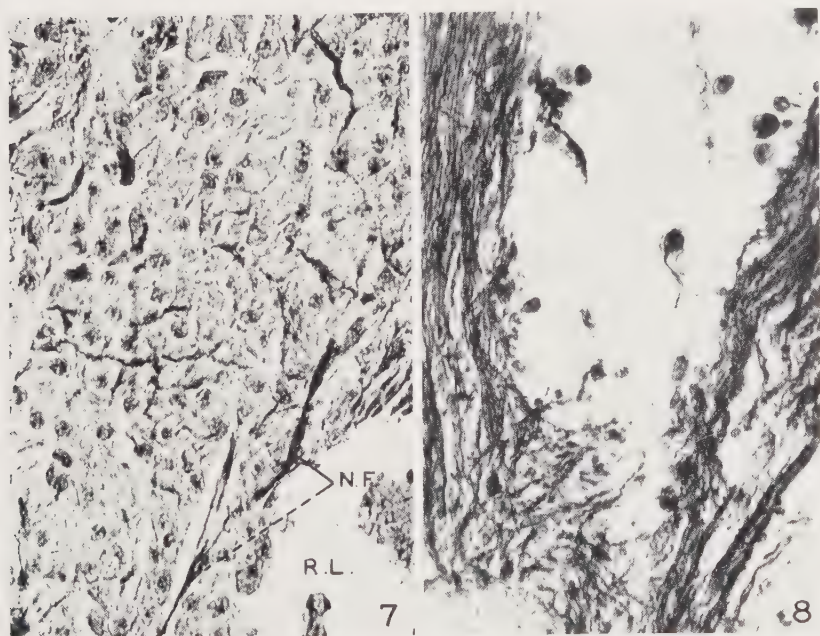
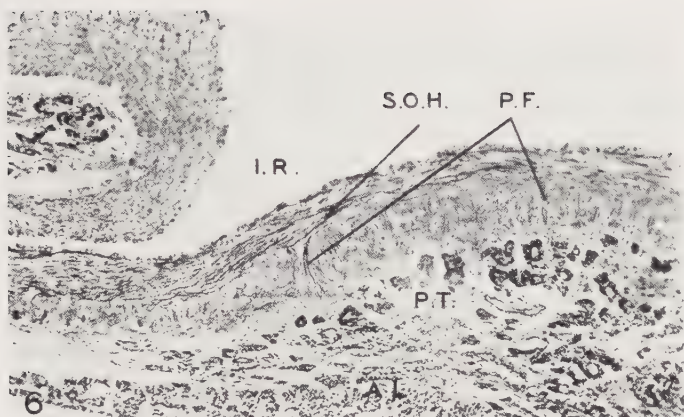


Fig. 6 Photograph of a part of the infundibular stalk and surrounding pars tuberalis. The figure shows a few of the penetrating fibers (P.F.) of the supra-optico-hypophyseal tract (S.O.N.) which definitely reach the pars tuberalis (P.T.). I.R., infundibular recess; A.L., anterior lobe. Bodian's silver method.  $\times 70$ .

Fig. 7 Photograph of the pars intermedia. Notice the relative abundance of nerve fibers (N.F.) and the prominent bundle adjacent to the residual lumen (R.L.). Pyridine silver.  $\times 410$ .

Fig. 8 Photograph of the pars nervosa showing a number of the bulb-like terminations of hypothalamic fibers. Pyridine silver.  $\times 410$ .



Hinsey and Markee ('33) and Marshall and Verney ('36) have suggested that it is this tract which carries the impulse from the central nervous system to the anterior hypophysis, thus bringing about ovulation as a response to copulation. Pertinent in this regard are the observations of Holweg and Junkmann ('32) which, although not above question, suggest that the cellular changes occurring in the anterior pituitary of castrated animals are brought about by a nervous connection which is parasympathetic in nature.

The writer's proof that removal of the superior cervical ganglion does not denervate the anterior lobe, is indirect evidence in favor of regarding Chorobski and Penfield's parasympathetic pathway as the possible course taken by impulses initiated in the secondary sex organs to pass to the anterior lobe. Verification is also given here for the observations of Chorobski and Penfield that removal of the cervical sympathetics does not materially reduce the number of intracranial nerve fibers along the vessels of the Willisian circle.

As an alternative hypothesis to explain the activation of the hypophysis to produce ovulation, Hinsey and Markee suggest that fibers from the hypothalamus may conduct impulses to the pars nervosa which in turn exerts an influence on the anterior lobe by hormonal transmission. In view of the independent circulation in the posterior lobe this theory has little to commend it. However, the present study has shown that occasional nerve fibers of hypothalamic origin do leave the stalk and pass directly to the anterior lobe. The diminutive size of this direct nervous connection and the failure to demonstrate it more consistently speaks against any justification for ascribing to it a functional importance. However, it may well be that one's inability to demonstrate these fibers consistently in all preparations is due to technical difficulties. Only further studies can answer this question. While not attempting an implication as to the importance of the fibers here observed, it can be stated that establishment of a functional nervous connection directly between hypothalamus and anterior lobe would go far in explaining those clinical and

experimental cases in which localized hypothalamic lesions produce evidence of marked sexual disfunction.

*Hypothalamic fibers in the pars nervosa and the pars tuberalis.* The growing belief that the pars nervosa is in some way associated with water metabolism imparts an unusual significance to the question of posterior lobe innervation. If the pituicytes, the characteristic cells of the pars nervosa, function as the secretory unit of an endocrine gland the importance of a nerve fiber connection between these cells and the hypothalamus can hardly be ignored. However, purely anatomical studies yield no very direct evidence for the existence of a significant nerve fiber-pituicyte relationship. The mere fact that the fibers in the pars nervosa ramify in areas occupied by large numbers of pituicytes cannot be considered as *prima facie* evidence of a trophic or functional control from the hypothalamus. On the other hand the confusing neurological picture in posterior lobe preparations makes the denial of such a control equally as insecure. In short, anatomical studies yield essentially no evidence on this point.

Our position is much the same regarding the significance of the hypothalamic fibers which pass to the pars tuberalis. Certain hypothalamic-pituitary syndromes have been explained by assuming the presence of such a nervous connection (Biggart, '35, '36). The evidence advanced in this study that such a fiber pathway actually exists is important in this connection, but it is not clear just what place this anatomical fact has in the explanation of pituitary hypothalamic relationship.

*The nerves of the pars intermedia.* The presence of nerve fibers in the intermediate lobe has been attested by many workers. Recently, however, Fisher ('37) has stated that in his experience the number of nerve fibers in the intermediate lobe of the cat is very small, and that they do not penetrate far into its substance. My own material, on the contrary, shows that the pars intermedia is well supplied by fibers and that these penetrate to all parts of the lobe. Indeed in figure 7 may be seen a fairly large bundle next to the residual lumen.

Since the part played by the *pars intermedia* in mammalian economy is little understood, no idea can be obtained as to the significance of its innervation. In the *Amphibia* and reptiles the *pars intermedia* functions in maintaining skin coloration. In those species which experience pigmentation changes in response to environmental stimuli there is apparently a nervous reflex mechanism operating either through the hypophysis or by more direct nervous connection with the pigment cells. Whether such a response is mediated by the autonomic or by fibers of the supra-optico-hypophyseal tract is not definitely known. This problem should be amenable to transplantation experiments.

#### SUMMARY

1. The hypophysis of the cat receives unmyelinated nerve fibers from the carotid plexus.

2. These fibers reach the gland by accompanying its arterial channels. Thus the fibers to the anterior lobe pass to it directly along vessels forming the direct vascular route, or indirectly along vessels which penetrate the *pars tuberalis* before reaching the anterior lobe. The fibers from the carotid plexus to the posterior lobe are few in number and accompany the arteries which enter that part from behind.

3. Since cervical sympathectomy does not entirely destroy these fibers it is apparent that they do not all arise from the superior cervical ganglion. It is suggested that some of them are derived from the glossopalatine nerve and are thus parasympathetic in origin.

4. The supraoptico-hypophyseal tract is the principle source of the nerve fibers in the *pars nervosa*. A considerable number of these fibers also penetrate into the *pars intermedia*.

5. A few fibers of the supraoptico-hypophyseal tract penetrate the infundibular stalk and reach the *pars tuberalis*. Occasionally fibers of similar origin can be traced to the anterior lobe.

6. Nerve fibers ramifying in the epithelial parts of the hypophysis (i.e., *pars intermedia*, *pars distalis*, and *pars*

tuberalis) have knob-like enlargements along their course which lie against the epithelial cells of the part. The pericellular networks of fibers described by Pines have not been demonstrated in this study.

7. Bulb-like terminations of the fibers of the supraoptico-hypophyseal tract which have been described in cattle and man are also present in the pars nervosa of the cat.

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# THE EFFECTS OF ESTROGENIC SUBSTANCES IN LEBISTES RETICULATUS (GUPPY) <sup>1</sup>

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TWO PLATES (TWELVE FIGURES)

While the literature concerned with the influence of hormones on the primary and secondary sex characters of mammals, birds and amphibians is voluminous, reports on such investigations in fish are less extensive. The effects of hormone treatment, in fish, on secondary sex characters such as nuptial coloring in the male and ovipositor lengthening in the female, have been reported by Tozawa ('29), Wunder ('31), Glaser and Haempel ('33), Fleishman and Kann ('32), Saphir ('34), Barnes et al. ('36), Owen ('36), and Kleiner et al. ('37). These reports, however, do not lend themselves to clear generalizations. Estrogenic and androgenic hormones in some instances seem to stimulate the same sex characters, and these responses again, are sometimes apparently not limited to sex hormones at all, but may be produced by a variety of substances. The effects of these substances on the gonads themselves, have been, as far as we are aware, largely ignored.

The experiments reported here were conducted for the purpose of observing the effects of estrogenic substances on the secondary sex characters and gonads of some readily available and easily handled fish.

<sup>1</sup> Submitted in partial fulfillment of the requirements for the Degree of Master of Science at New York University. A preliminary report of this work has been made (Berkowitz, '37).

*Lebistes reticulatus* (guppy), an ovoviviparous form, was chosen for this study. A single mating may furnish four or five litters. The adult male *Lebistes* possesses, as its secondary sex characters, a gonopod or intromittent organ (a modified fin) and various color patterns. The adult female possesses no gonopod or coloring but is much larger than the male and, in addition, has a definite 'gravid spot.' These differentiating sex characters are not present at birth, at which time both male and female are identical in appearance. At about 35 days after birth, there is a divergence in size of the two sexes, from which time the females normally become larger than the males. In the male, the gonopod is first noticeable at approximately 40 days, while the colorations appear from 50 to 60 days after birth.

The genetics of this form has been described by Schmidt ('19), and Winge ('22, '22 a, '23, '27, '30). The spermatogenesis has been studied by Vaupel ('29). Dildine ('33, '36) and Goodrich et al. ('34) discussed the histology of the embryonic gonads of both sexes. The latter investigator also gave an account of the histology of the adult gonads and correlated these with the appearance of the secondary sex characters. Blacher ('26) studied a number of naturally occurring sex abnormalities in *Lebistes*, and concluded that male coloring was dependent on the testicular hormone.

The author wishes to acknowledge the helpful criticism of Dr. Robert Gaunt, under whose direction this work was undertaken, as well as the advice and suggestions of Drs. Harry A. Charipper and Morris H. Harnly.

#### MATERIALS AND METHODS

The fish utilized in the present investigation were kept in 2-gallon aquaria, each containing not less than four nor more than eight animals. During the winter months, the temperature of the water was thermostatically controlled at approximately 78°F. The estrogenic substance here given was in the form of progynon tablets containing 45 rat units of

estrone and estriol.<sup>2</sup> One tablet was crumbled into each tank three times per week. The fish ate the tablets readily. The dimorphic secondary sex characters studied were size, shape, coloration, condition of anal fin, and the presence or absence of the 'gravid spot.'

Eighty-four fish were hormone treated. Of this group, seventy-four were fed on the hormone from birth for periods of 1 to 5 months. A number of such treated animals were sacrificed for histological observations after varying periods of treatment. In some animals treatment was discontinued after 1 or 2 months, but the fish allowed to live 5 to 10 months to determine the permanence of the effects induced by treatment. After this period, these animals were sacrificed and their gonads prepared for histological examination. The remaining ten animals were males subjected to treatment after they had reached sexual maturity. They were fed on the hormone for periods of 6 weeks to 4 months.

Fifty-five litter-mate controls were raised and handled in a manner identical with the experimental group, except that no hormone feeding was employed. Histological sections of the gonads of sixteen male and nine female controls served as a standard for comparison with those of the treated animals. No spontaneous abnormalities in either secondary sex characters or gonads of controls were observed.

#### EXPERIMENTAL RESULTS

##### *A. Effects on the secondary sex characters*

None of the seventy-four animals treated from birth showed male secondary sex characters during the period of treatment. Forty-five of these fish had reached a growth stage and age at which sexual differentiation should have been completed or at least clearly indicated. The size and shape of such animals approached that of normal females. Their body colorations and the condition of their anal fins were in all

<sup>2</sup> The author is indebted to Dr. Gregory Stragnell of the Schering Corporation for supplying the progynon; to Mr. C. H. Breder of the New York Aquarium; and to Mr. H. E. Potts for animals and advice as to their proper care.

respects characteristically female. All fish treated for 3 months or over also developed gravid spots.

Seven of the fish treated from birth were fed on hormone for 1 month and then placed into tanks containing no hormone. One assumed male secondary sex characters 2 weeks after discontinuance of the hormone treatment. Another took 4 months to acquire a gonopod and the dimorphic colorations of the male. During this time, however, this latter animal had grown to the size and shape characteristic of the female. In addition, it had acquired a definite gravid spot, which gradually disappeared only after the anal fin was transformed to a gonopod and the male colorations were well established. The female size and shape was retained for 11 months when the animal was sacrificed. At that time, it measured 29 mm. from tip of snout to base of caudal fin. The other five fish of this group were apparently genetic females, and showed no signs of having been affected by the hormone treatment. They matured and reproduced normally.

Fourteen of the remaining fish were treated for 2 months. At the end of this period, all showed only female secondary sex characters. Hormone treatment was then discontinued, and eight of these animals developed a gonopod, assumed male color and male sex behavior, approximately 40 days after the discontinuance of the hormone, i.e., a period exactly the same as the time necessary for normal males to attain sexual differentiation from birth. The size and shape of these fish were, however, intermediate between that of normal males and females. The average length of such reverted males was 26 mm. as compared to 18 mm. for control males, and 30 mm. for control females. The other six fish were apparently genetic females that were not modified by the treatment.

The rest of the group (fifty-three fish), treated from 1 to 5 months after birth, were sacrificed for histological study at the time treatment was discontinued.

The secondary sex characters of the ten adult males could not be significantly modified by estrogenic treatment, continued for periods up to 4 months. All such animals retained

their full complement of colors, their well-developed gonopod, and their libido. They did not grow beyond the size characteristic of their sex, nor did they develop a gravid spot.

### *B. Effect on gonad structure*

In agreement with the description of Goodrich et al. ('34), the normal development of the male gonad, as illustrated by our control animals, was briefly as follows: Before the appearance of male secondary sex characters there was a continual proliferation of primary spermatogonia which tended to aggregate into nests of cells. As development proceeded these nests became surrounded by a stroma of connective tissue forming the cysts typical of mature males. At the time of transformation of the anal fin into an intromittent organ, all stages of spermatogenesis, with the exception of mature sperm, were found in the gonads (figs. 1 and 5). It was only when the color typical of the male sex was definitely established that mature sperm were also found (figs. 3 and 6).

The male gonad showed two tendencies as a result of hormone treatment begun at birth, and continued for a period of 3 months. In one group there was apparent not only an inhibition of spermatogenesis but a definite tendency toward involution of the gonad (figs. 2 and 4). In a second larger group, there was apparent, under treatment, a development of female gametic characteristics in addition to the suppression of spermatogenesis (figs. 10, 11 and 12). In both groups, there appeared an increase in the number and size of the sperm ducts, with a stratification of the lining cells and an accumulation of colloid in the lumina. There seemed to be a direct stimulation of these structures by the hormone.

In general, the gonads of the group bearing ovotestes were definitely more compact than that of a normal testis of the same age and size (compare fig. 12 with fig. 3). The right and left gonads failed to fuse throughout the median plane. The ovarian areas were not localized in any specific region of the gonad. They were characterized by the presence of large rounded cells containing a vesicular nucleus whose



chromatin showed nucleolar condensations. The karyosomes of these cells were found, usually, centrally located in a goodly amount of vacuolated cytoplasm containing yolk granules. Enclosing some of these oocytes and many well-rounded cells considered gametogonia, was a definite connective tissue capsule (fig. 10).

In general there appeared to be no effect of estrogenic substances on the female gonad treated from birth for periods up to 5 months. A further study is being made of this point.

An examination of the male gonads of animals treated after sexual maturity, presented in some instances features which, with a few exceptions, were similar to those described above. The gonad was larger, but showed the same tendency toward compactness as previously described (fig. 9, compare with fig. 3). In these cases there had been a definite fusion, in the median plane, of the right and left testes. This fusion was, of course, the result of normal development, and is not, therefore, to be considered as an effect of hormone treatment.

A closer examination of sections of gonads of this type showed areas containing definite ovarian tissue close to areas of clearly defined spermatogenic tissue (figs. 7 and 8). While a majority of the egg cells were localized in the marginal third of the gonad, an occasional area of ovarian tissue was found in the mid-medullary region. These egg cells were large rounded units which compared favorably with normally maturing ova. The nuclei of these cells were vesicular, well differentiated, and contained clearly defined nucleoli. Cytologically, however, the characteristic peri-nuclear hyaline zone was not always apparent. Around most of the larger egg cells were aggregates of smaller gametogonia, apparently ovarian in nature (fig. 8).

The connective tissue stroma throughout the gonad appeared to be hypertrophied and made up of well-defined connective tissue cells. This stroma was unlike that usually found in the male gonad, but was strikingly similar to that characteristic of normal ovaries. Such gonads further showed a definite decrease in the number of cysts containing mature

sperm (fig. 9). A good number of the cysts contained gametocytes in different stages of spermatogenesis. The nuclei of these gametocytes appeared to be degenerating.

Sixteen normal male gonads were carefully examined and none showed any such ovarian tissue.

The gonads of five males treated for 1 or 2 months, beginning at birth, and sacrificed 5 to 10 months after discontinuance of hormone treatment, showed no regions of ovarian tissue, and with one exception, the gonads appeared normal.

#### DISCUSSION

The effect of estrogenic hormone on the secondary sex characters is clear. Excessive doses of such substances administered to genetic males, inhibited the development of male secondary sex characters and caused the development of female secondary sex characters. The effects on the gonads are, however, only in part compatible with the effects of such substances in higher forms. In those fish in which estrogenic hormone treatment caused only testicular inhibition, a situation comparable to mammals might be postulated, i.e., that the hormone exerted its effect through an hypophyseal inhibition (Moore and Price, '32). This interpretation will not, however, adequately explain those cases in which the hormone suppressed testicular activity but stimulated (or released from inhibition) ovarian elements in the male testis. The apparent absence of effect of the hormone upon the female gonad is also not compatible with the idea that a marked hypophyseal inhibition accounted for its effects in the male. The implication is therefore, that in fish, unlike in the mammal, estrogenic hormone acts directly on the male gonad and not by way of the hypophysis. A further analysis of this question is in progress.

In amphibians, parabiotic and gonad transplantation experiments have given results somewhat similar to those found here. That this is questionably, if at all, due to gonadic hormones appears from the work of Witschi and colleagues (Witschi, '34, '37) who attribute the effects to other agents.

Work on birds has given results somewhat more directly comparable to those reported here. Estrogenic hormones have been shown to cause the formation of an hermaphroditic gonad when injected into embryonic male chicks. These same substances, however, had no significant effect on the embryonic female chicks (Kozelka and Gallagher, '34; Willier et al., '35; Wolff and Ginglinger, '36; and Willier et al., '37). Male urine extracts also produced an ovotestis in the male, but had no effect on the female gonad (Willier et al., '37). Willier ('37) has since shown that synthetic androsterone and dehydroandrosterone produce ovotestes in both male and female embryonic chicks, while testosterone propionate has a similar effect on the female only.

The male gonad of *Lebistes* is apparently still more responsive to estrogenic treatment than that of birds, for here even on the gonads of males in which sexual differentiation was already complete, an ovotestis has been produced as a result of hormone treatment.

Since this report was completed we learned of the work of Padoa ('37) who obtained somewhat similar results with estrogens in young trout. Witschi and Crown ('37) reported that *Xiphophorus helleri* females, treated with testosterone propionate, showed male secondary sex characters and inhibited ovaries. Eversole (unpublished) in our laboratory has made similar observations on testosterone-treated *Lebistes*.

#### SUMMARY

1. Estrogenic hormone fed to male guppies from the time of birth completely prevented the development of any male secondary sex characters and caused the assumption of female secondary sex characters. Males treated from birth for 1 or 2 months assumed male secondary sex characters 40 days after estrogenic treatment was stopped. Estrogenic hormone fed to guppies after sexual differentiation was complete, had no appreciable effect on the secondary sex characters.

2. Estrogenic hormone treatment started at birth and continued for 2 months partially suppressed spermatogenesis in the testes, but apparently stimulated growth of sperm ducts. If the treatment was continued for 3 months, there was, in some instances, a development of an ovotestis in addition to the inhibition of spermatogenesis. Estrogenic hormone treatment started after sexual maturity had been reached and continued for 6 weeks likewise caused the testes to become hermaphroditic.

3. The apparent inhibition of the male gonad both in hormone elaboration and spermatogenesis may be due to a direct effect of estrogenic hormone on the gonad, rather than an hypophyseal inhibition as in mammals.

4. There was no effect of estrogenic hormone in females on secondary sex characters, and probably none on the ovaries.

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## PLATES

## PLATE 1

### EXPLANATION OF FIGURES

1 Cross section of testis of normal male at time of transformation of anal fin into an intromittent organ, showing all stages of spermatogenesis with the exception of mature sperm.  $\times 160$ .

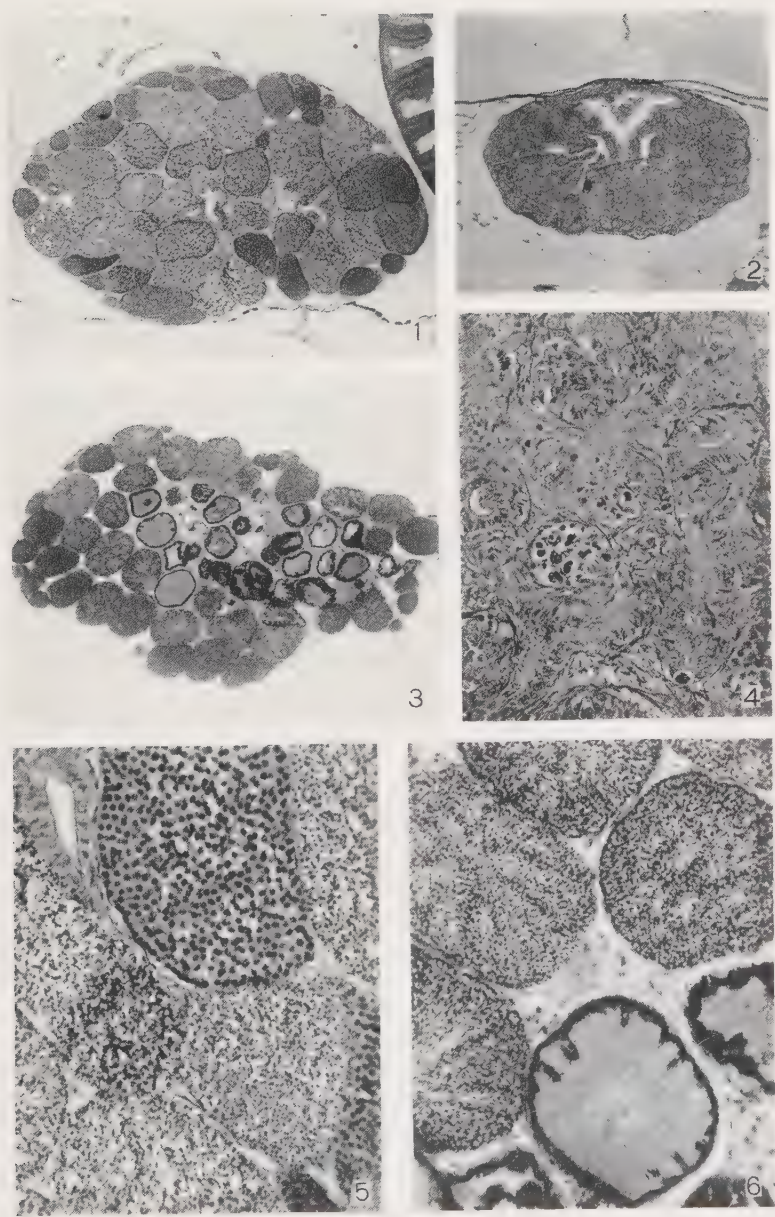
2 Cross section of testis of a 3-month-old genetic male fed on hormone from birth for a period of 3 months, showing a degenerate and involuted gonad.  $\times 160$ .

3 Cross section of testis of normal, sexually differentiated male, age 3 months, showing all stages of spermatogenesis including cysts of mature sperm.  $\times 160$ .

4 Same as figure 2, showing a marked inhibition of spermatogenesis.  $\times 800$ .

5 Same as figure 1.  $\times 800$ .

6 Same as figure 3, showing, in more detail, a cyst of mature sperm.  $\times 800$ .



## PLATE 2

### EXPLANATION OF FIGURES

7 Same as figure 9, showing a well-developed egg (upper right), a cyst of mature sperm (upper left), a cyst of spermatogenic cells (lower right), and a number of gametogonia (upper right).  $\times 800$ .

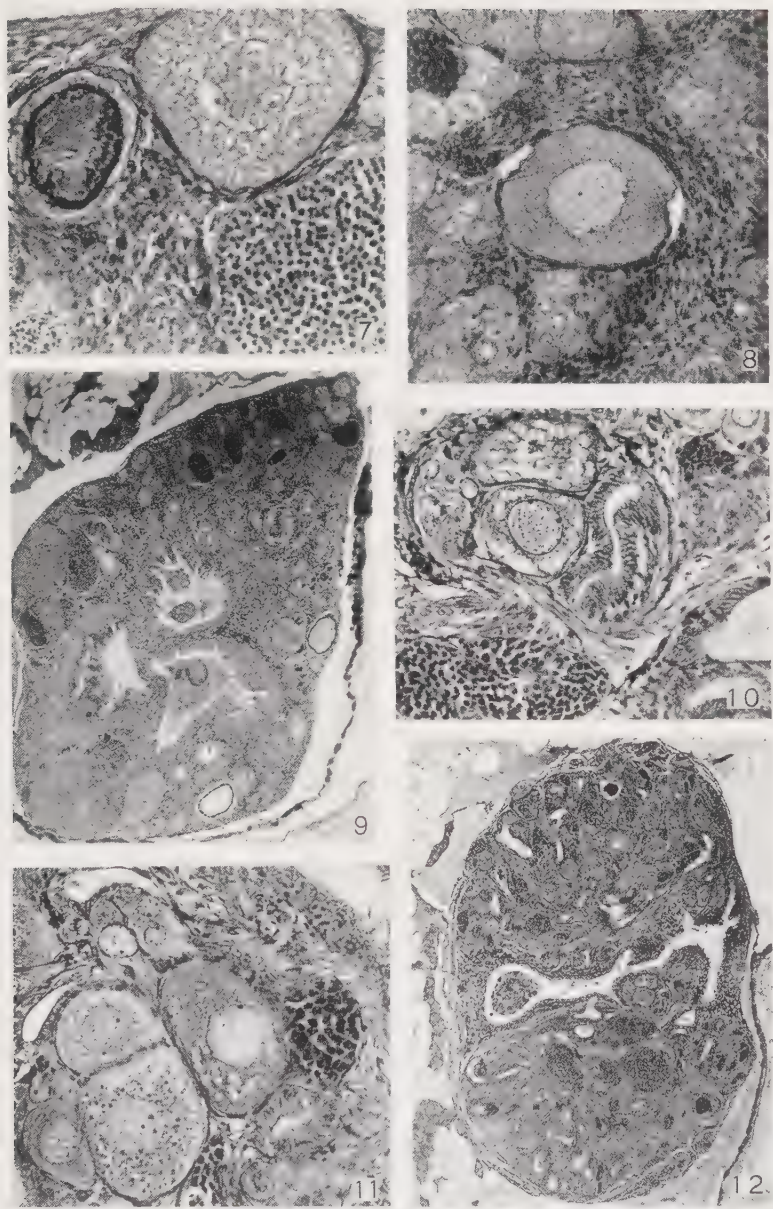
8 Same as figure 9, showing a well-developed egg (center), a large number of gametogonia, and a small area of degenerating spermatogenic cells (upper left).  $\times 800$ .

9 Cross section of ovotestis of adult male treated with estrogenic hormone beginning at sexual maturity and continued for 6 weeks, showing a compact gland containing all stages of spermatogenesis and a number of well-developed eggs. (Compare with fig. 3.)  $\times 160$ .

10 Same as figure 12, showing an ovarian area (upper center) lying in close proximity to a testicular area (lower left).  $\times 800$ .

11 Same as figure 12, showing a nest of eggs and degenerating spermatogenic cells (right center).  $\times 800$ .

12 Cross section of ovotestis of a 3-month-old genetic male fed on estrogenic hormone from birth for a period of 3 months. The gonad is very compact in contrast to the loose condition of the testis of a normal male (compare with fig. 3).  $\times 160$ .







## BILATERAL DOUBLE INFUNDIBULUM OF THE \ UTERINE TUBE <sup>1</sup>

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ONE FIGURE

Developmental anomalies in the ducts which form part of internal generative organs of the female are common indeed. Among the frequent variations is the occurrence of accessory abdominal openings, two or even three in number, which may lie near the fimbriated extremity or at some distance from the latter along the uterine tube. Common, also, are cystic enlargements, derived from portions of the wolffian and müllerian ducts of the embryo.

Not frequent are cases of bilaterally placed tubal anomalies which are accompanied by cysts both numerous and prominent. In this category belongs the case which we present herewith.<sup>2</sup>

The uterine tube of each side, in our specimen, terminated in a double infundibulum (fig. 1). The paired infundibula, both of them patent, joined to form a common duct after traversing a distance of approximately 1.5 cm. Both right and left tubes were of normal length.

<sup>1</sup> From the Departments of Gynecology and Anatomy, Northwestern University Medical School, Contribution no. 262 from the latter.

<sup>2</sup> The tubes were removed with the uterus in a hysterectomy from a woman 45 years of age; the uterine wall contained leiomyomata of large size. The uterus itself was large, being 12.5 cm. long, 7.5 cm. wide. The left ovary was entirely normal, the right somewhat cystic.

On the left side occurred three pedunculated cysts, one of large, and two of small, size. The two small cysts were attached, each by a long thin stalk, to the uterine tube near its fimbriated extremity; they were assumedly hydatids of Morgagni.<sup>3</sup> The large single parovarian cyst, of grape-like size and form, arose from the posterior surface of the mesosalpinx by a heavy stalk which itself carried multiple small cysts. Additional cysts occurred within the mesosalpinx of the left side; like the large stalked element just described they were regarded as being tubes of Kobelt.

On the right side the mesosalpinx was free from cysts; but six, of the pedunculate variety, occurred at the extremity of a cord-like stalk which was itself a prolongation from the dorsal leaf of the broad ligament.

<sup>3</sup> Since the entire preparation was retained as a museum specimen, it was not possible to determine, histologically, the relation of the cysts to the ducts of the epoöphoron.

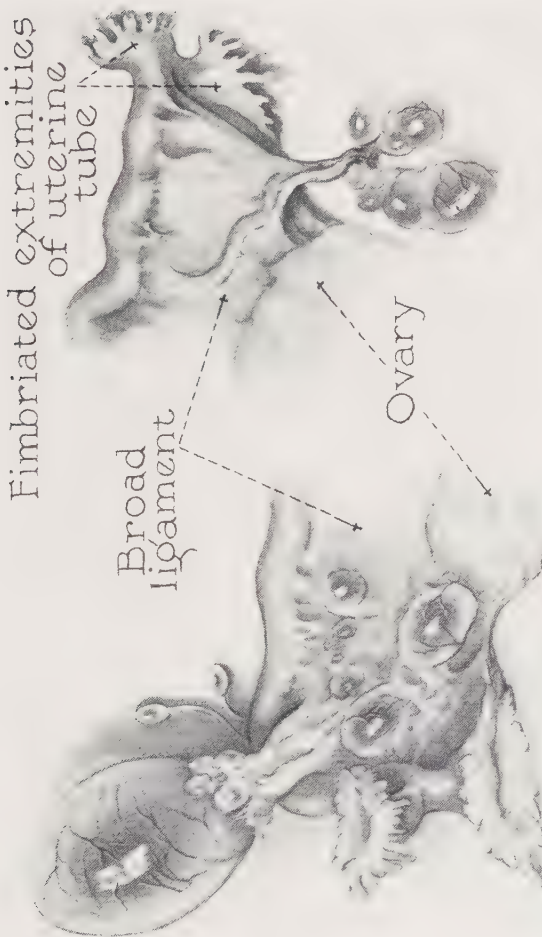


Fig. 1 Posterior aspect of the mesosalpingeal portion of the broad ligament, with associated structures; left and right sides. Each half includes the uterine tube, ending in double abdominal ostia, the mesosalpinx, the attached margin of the ovary, pedunculated cysts (elevated on left side to reveal cysts within the broad ligament). Natural size; drawing from fresh specimen.





# A CASE OF CONGENITAL CLUBHAND WITH A REVIEW OF THE AETIOLOGY OF THE CONDITION

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EIGHT FIGURES

## INTRODUCTION

There are two varieties of clubhand, the congenital and the acquired. The acquired type develops as the result of an injury to the epiphysis at the lower end of the radius or ulna with subsequent defective growth of the affected bone, the hand in consequence, being deviated to one or other side. The congenital variety may consist in deviation to the radial or ulnar side; dorsal, or palmar flexion.

The congenital cases may be primarily congenital, i.e., due to some developmental defect. These cases are often hereditary and are associated with partial or complete absence of one of the bones of the forearm. Blencke reported the condition in four children in one family and Joachimsthal similar deformities in a mother and five children. The secondary congenital deformity is one where the fetus has suffered from no inherent developmental defect but the deformity has resulted from disease of the fetus or external pressure applied to the fetus (e.g., oligo hydramnios). In this case the bones of the forearm may be normal or curved.

From a study of the literature of the primary congenital cases it has been found that defects of the radius are most common. It is more usual to find the condition unilateral than bilateral and for every four cases in which the radius is totally absent there is only one case with part of the radius present.

According to Antonelli ('05) 60% of the cases are males. Kato ('24) maintains 40% are males and that the number of unilateral and bilateral cases is almost equal.

Since the first authentic report of a case of this condition by Petit (1733) between 250 and 300 cases have been put on record. However, owing to the fact that congenital clubhand in itself does not necessarily shorten the life of the individual, many of these cases have only been subjected to clinical and radiological investigation. It may therefore be of value to record a case where it has been possible to dissect the affected extremity, and where several variations not previously described have been found.

#### DESCRIPTION OF SPECIMEN

A fetal monstrosity consisting of two average-sized full time infants was sent to the anatomy department of Aberdeen University by Dr. James S. Stewart. The monster consists of two male infants lying with the ventral surfaces opposed and joined together by thorax and abdomen. There are separate heads and upper and lower limbs but the thorax and abdomen are common to both. The mother was a healthy woman aged 35 years, who had had two previous normal pregnancies, one 5 years and the other 10 years previously. There was no history of congenital malformations in the family and during this particular pregnancy there had been no systemic upset of any kind.

The left upper limb of one fetus exhibits a congenital clubhand deformity. The left lower limb of the same fetus has only two digits present, namely, the great and second toes. The remaining three metatarsals are missing in addition to the digits, but situated halfway along the lateral border of the foot there is a cutaneous appendage representing a third toe.

#### *External examination*

The shoulder joint of the affected limb is normal in external appearance and movements. The humerus on this side is 7 mm. longer than that on the right side and apart from the

fact that its lower end is slightly broader between the epicondyles than on the right side, presents no other abnormality.

Movements at the elbow joint are confined to about  $10^{\circ}$  of flexion and the olecranon process projects posteriorly and is



Fig. 1 Drawing of the dorsolateral aspect of the undissected limb.



Fig. 2 Radiogram of the undissected limb.

unduly prominent. The shaft of the ulna can be felt to be curved posteriorly and to be considerably thickened and the lower end of that bone projects subcutaneously at the distal end of the limb. The hand is deflected outward so that its

radial border lies in apposition with the radial border of the forearm the fingers pointing toward the shoulder. Four digits are present, the thumb being absent and syndactylism of the index and middle fingers is present, the web extending distally to the level of the middle phalanges. The terminal phalanx of the index finger is flexed toward the middle finger and an abnormal degree of mobility of this phalanx is noted.

There is a cutaneous bridge connecting the anterolateral surface and radial border of the forearm with the palm and radial border of the hand. The bridge extends on the palm in the line of the shaft of the third metacarpal to a point just short of the head of the bone but as it passes to the radial border of the hand the distal edge of the bridge runs obliquely so that the bridge from the radial border of the hand to the radial side of the forearm is shorter than that on the palmar aspect.

#### *X-ray examination*

Anteroposterior (fig. 2) and the lateral views of the affected limb have been taken. The humerus shows no abnormality apart from unusual breadth of the lower end. The upper and lower epiphyses have not started to ossify. The ulna appears to be backwardly displaced on the humerus at the elbow joint and the shaft of the ulna is thickened and bowed posteriorly. Four normal metacarpals are present. The three medial digits contain three phalanges but the second phalanx of the index finger is missing.

No centers of ossification are present in the carpal bones or in the lower or upper ends of the ulna.

#### *Dissection of muscles*

The deltoid is well developed and converges abruptly to be inserted into a rough impression on the lateral surface of the shaft of the humerus at the junction of the upper third and lower two-thirds. The muscle at its insertion is tendinous and sends a slip to the lateral head of the triceps (fig. 4).

The coraco-brachialis and short head of the biceps arise as a single fused muscle mass from the tip of the coracoid process. The long head of the biceps arises as usual from the supraglenoid tuberosity and pierces the capsule of the shoulder joint. The coraco-brachialis, short and long heads of the biceps are fused into a single muscle mass just below the surgical neck of the humerus and this composite muscle

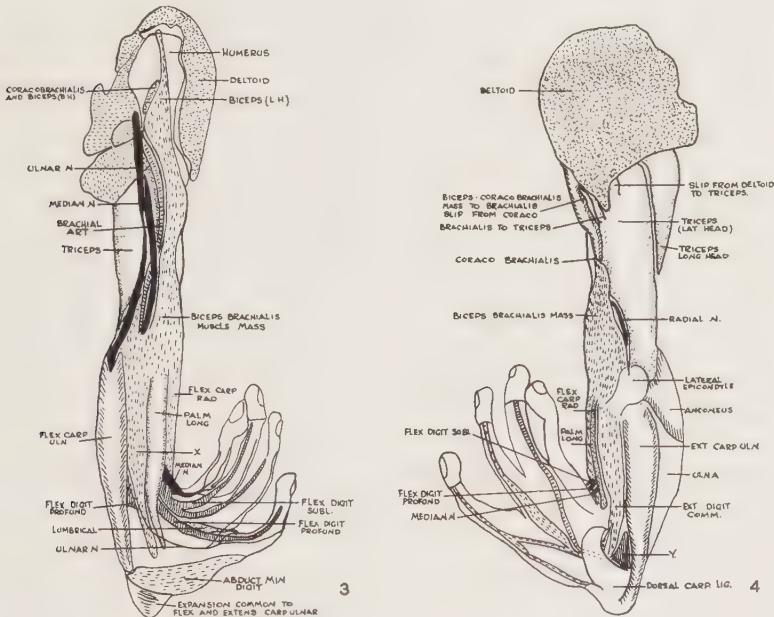


Fig. 3 Drawing of dissected limb showing the superficial structures on the volar aspect.

Fig. 4 Drawing of dissected limb showing the superficial structures on the dorsal aspect.

divides again into three bellies at the junction of the upper third and lower two-thirds of the shaft of the humerus. The lateral head passes round the lateral aspect of the shaft of the humerus and fuses with the lateral head of the triceps (fig. 4). The intermediate one is inserted halfway down the anterior aspect of the shaft of the humerus and becomes continuous with a muscle mass attached to the anterior aspect of



the lower half of the shaft of the humerus (figs. 3, 4). This mass is triangular in shape, broad below and narrow above, the upper end lying in close proximity to the insertion of the deltoid. This is almost certainly a mass resulting from fusion of the biceps and brachialis.

The medial of the three heads resulting from the fusion of the biceps and coraco-brachialis is inserted halfway down the medial surface of the shaft of the humerus (fig. 4). This position corresponds to a normal coraco-brachialis but from its point of insertion a fibro-muscular slip extends down to the medial epicondyle of the humerus.

The brachialis is attached to the lower half of the anterior aspect of the shaft of the humerus and below covers the bone from the medial to the lateral epicondyles. The body of the muscle is directly continuous below with the origins of the flexor carpi radialis, flexor carpi ulnaris, palmaris longus and extensor digitorum communis.

The triceps is normal in all respects but its lateral head is joined by a slip from the deltoid and one from the coraco-brachialis biceps mass (fig. 4).

The muscles of the forearm exhibit greater abnormalities than those of the arm. The pronator teres appears to lie deep in the forearm because of the continuity of the biceps-brachialis muscle mass with the palmaris longus and flexor carpi radialis. It arises by two heads, one from the anterior aspect of the medial epicondyle and the deep head for the front of the upper part of the anterior surface of the shaft of the ulna. Between the two heads the median nerve and ulnar artery pass. The muscle is inserted into the lateral side of the shaft of the ulna about its middle third and from its lower end a tendinous slip is continued down to the lower end of the bone (fig. 6).

The flexor carpi radialis arises as a continuation of the biceps-brachialis mass on the lower end of the humerus. It extends down the front of the forearm and is inserted into the base of the first metacarpal bone (i.e., that of the index finger) (fig. 3).

Lying on the medial side of the flexor carpi radialis is a muscle which arises as a continuation of the biceps-brachialis mass and running down the forearm on the ulnar side of the flexor carpi radialis is inserted by a narrow tendon into the pisiform and the hook of the hamate. From its position and direction this muscle may be assumed to be the palmaris longus though its tendon passing deep to the palmar fascia to an insertion into the carpus is very unusual.

The flexor carpi ulnaris arises from the medial epicondyle of the humerus, from the medial side of the olecranon process, and from the upper two-thirds of the dorsal border of the ulna. It ends in a tendon which is inserted into the pisiform and sends an expansion round the lower end of the ulna where it fuses with the extensor carpi ulnaris and also with the dorsal carpal ligament. The ulnar nerve passes from the arm to the forearm between the humeral and ulnar heads of the muscle (figs. 3, 5).

The flexor digitorum sublimis arises from the front of the medial epicondyle of the humerus and also from the anterior aspect of the upper fourth of the shaft of the ulna. The muscle descends in the forearm anterior to the flexor digitorum profundus with the median nerve at first on its deep surface and then lateral to it. The tendons of the muscle appear between the flexor carpi radialis and palmaris longus and run to the terminal phalanges of the middle and ring fingers (fig. 3).

The flexor digitorum profundus arises from the anterior and lateral aspects of the shaft of the ulna in its upper two-thirds as a stout fleshy muscle. It descends behind the flexor sublimis and the median nerve and emerges above the wrist on the medial side of the median nerve between the flexor carpi radialis and the palmaris longus to end in a single stout tendon which tranverses the palm and passes to its insertion to the base of the terminal phalanx of the little finger. There is a single lumbrical muscle arising from its radial side in the palm which passes round the base of the proximal phalanx of the little finger and fuses with the extensor tendon of that finger (figs. 3, 5).

There is thus only one flexor tendon to each of the three medial digits and it runs to the base of the terminal phalanx. There are no flexor or extensor tendons to the index finger.

Lying on the medial side of the flexor digitorum sublimis and profundus and anterolateral to the flexor carpi ulnaris there is a muscle which springs from the anterior surface of the upper third of the shaft of the ulna by a narrow flat tendon on the medial side of the flexor digitorum sublimis. The

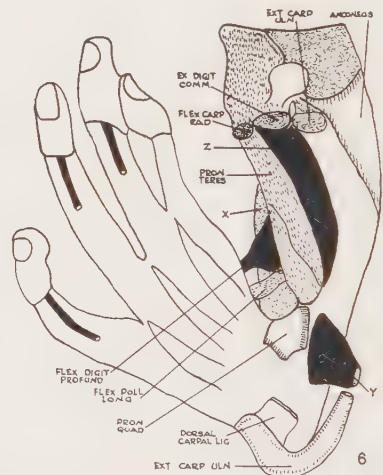
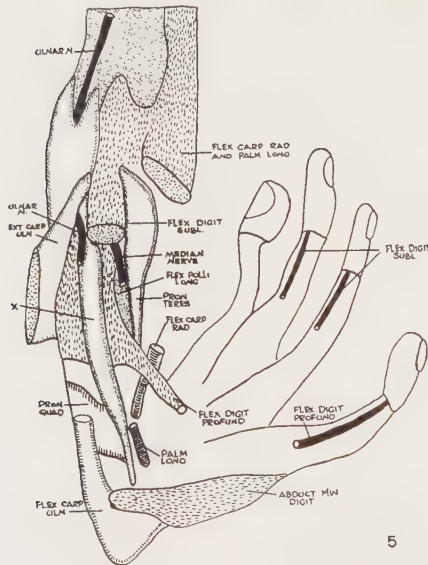


Fig. 5 Drawing of the dissected limb showing the deep structures on the volar aspect.

Fig. 6 Drawing of dissected limb showing the deep structures on the dorsal aspect.

muscle origin extends up to the coronoid process and is fused with the anterior aspect of the capsule of the elbow joint. The tendinous origin expands into a muscular belly which ends in a tendon below and is inserted into the anterior aspect of the capsule of the wrist joint and through it to the proximal row of carpal bones.

This muscle (x in diagrams) may be the upper medial part of the flexor digitorum profundus which has become separated

from the lower lateral part of the muscle. As the lower lateral part of the muscle has been displaced medially to become inserted into the little finger this medial portion has been inserted into the carpus (figs. 3, 5).

Between the pronator teres and flexor digitorum profundus a muscle arises from the anterior aspect of the upper third of the shaft of the ulna. This muscle ends in a short tendon inserted into the radial side of the base of the metacarpal bone of the index finger. This probably represents the flexor pollicis longus which has been inserted into the index finger owing to the absence of a thumb (figs. 5, 6).

Lying on the lateral side of the pronator teres there is a muscular slip which is attached continuously from the distal extremity of the humerus down the lateral edge of the anterior surface of the ulna. It tapers gradually and below it consists of a flat tendinous expansion which extends down to the junction of the middle and lower thirds of the ulna. In its upper part the muscle is fused with the pronator teres. This muscle (z in the diagram) may be part of the extensor carpi radialis brevis (fig. 6).

Medial to the lower end of the pronator teres and arising from the anterior aspect of the lower half of the shaft of the ulna there is a muscle which is inserted into the dorsum of the radial side of the carpus by a musculo-tendinous expansion. This represents the pronator quadratus and when it had been divided the hand could be rotated so that its axis lay in line with the axis of the ulna (fig. 6).

The brachioradialis and the extensor carpi radialis longus are missing.

The extensor digitorum communis arises from the anterior aspect of the lateral epicondyle of the humerus and from the common biceps-brachialis mass. The muscle runs down the lateral side of the forearm and above the wrist divides into two tendons which pass under the dorsal carpal ligament and are inserted into the little and ring fingers. From the tendon passing to the ring finger a very fine tendinous slip passes over to fuse with the tendon to the little finger. This represents the extensor minimi digiti tendon (fig. 4).

From the anterolateral aspect of the lower third of the shaft of the ulna a muscle arises from a linear origin. It passes downward and narrowing rapidly passes under the dorsal carpal ligament. It gives rise to a tendon which is inserted like an extensor tendon into the middle finger. From its origin, direction and position this muscle (Y in diagrams) probably represents the extensor indicis proprius (figs. 4, 6). The anconeus presents no abnormality.

The extensor carpi ulnaris arises from the lateral epicondyle of the humerus, from the dorsolateral surface of the ulna below the anconeus, and also from the dorsal border by an aponeurosis common to it and to the flexor carpi ulnaris. The muscle runs downward to the lower end of the ulna where it fuses over the lower end of the bone with the flexor carpi ulnaris. It also fuses with the dorsal carpal ligament and sends a slip to be inserted into the base of the fifth metacarpal bone (fig. 4).

The supinator, abductor pollicis longus, extensor pollicis longus and extensor pollicis brevis muscles are all absent. The hypothenar muscles are normal but the muscles of the thenar eminence are absent. There is, however, a small muscle arising from the os magnum which is inserted into the radial side of the shaft of the metacarpal of the index finger. This may represent an aberrant opponens pollicis muscle. There are three dorsal and three palmar interosseous muscles. There is no dorsal interosseous muscle of the index finger.

### *Arthrology*

The elbow joint is formed by articulation of the coronoid and olecranon processes of the ulna with the trochlea of the humerus. There are two joint cavities, one between the trochlea and the coronoid process and a second between the olecranon process of the ulna and the olecranon fossa on the lower end of the humerus. This fossa is large, flat and shallow, and extends some distance up on the posterior aspect of the lower end of the humerus. There are two distinct synovial cavities and there is a tough ligament extending from the



posterior end of the trochlear surface to the base of the olecranon process and attached laterally and medially to the inside of the joint capsule. This ligament is covered by synovial membrane on both sides. The capsule of the upper joint contains a considerable quantity of fat.

The trochlear surface of the humerus is smooth and shining as is the articular surface of the coronoid process. The olecranon fossa and the articular surface of the olecranon process are, however, covered by a thin membrane continuous with the periosteum and this gives those areas a dull appearance. The coronoid fossa is very shallow and filled with the attached edge of the joint capsule. The medial epicondyle is of normal shape and size but the lateral one is fused with the capitulum into a single bony mass. This capitulum is not covered with articular cartilage and the capsule of the joint is closely adherent to it. The joint capsule is attached round the edge of the olecranon and coronoid processes and above to the edge of the trochlea anteriorly, medially and laterally while posteriorly it passes upward and skirts the border of the olecranon fossa.

Flexion at the joint is practically impossible because of the tightness of the capsule passing from the tip of the olecranon process to the border of the olecranon fossa and also because of the presence of the ligament dividing the joint into two separate cavities and stretching from the posterior border of the trochlea to the base of the olecranon process.

The wrist joint is formed by articulation of the anterior surface of the lower end of the ulna with the lunate, triquetral and pisiform bones. There is not a true joint cavity, the bones being bound together by tough fibrous tissue and the bony surfaces not being covered by smooth shiny articular cartilage.

### *Osteology*

The humerus is 7 mm. longer than the corresponding bone of the opposite side. The shaft is slender and the extremities appear large in proportion to the thickness of the shaft. The bicipital groove is poorly marked and the capitulum is fused with the lateral epicondyle.

The ulna is much shorter than the corresponding bone of the opposite side but is thickened and bowed posteriorly. The upper half is the portion of the bone that has become bowed more than the lower half. There is a rounded anterior border, a sharp posterior border and broad medial and lateral surfaces. The coronoid and olecranon processes are normal and

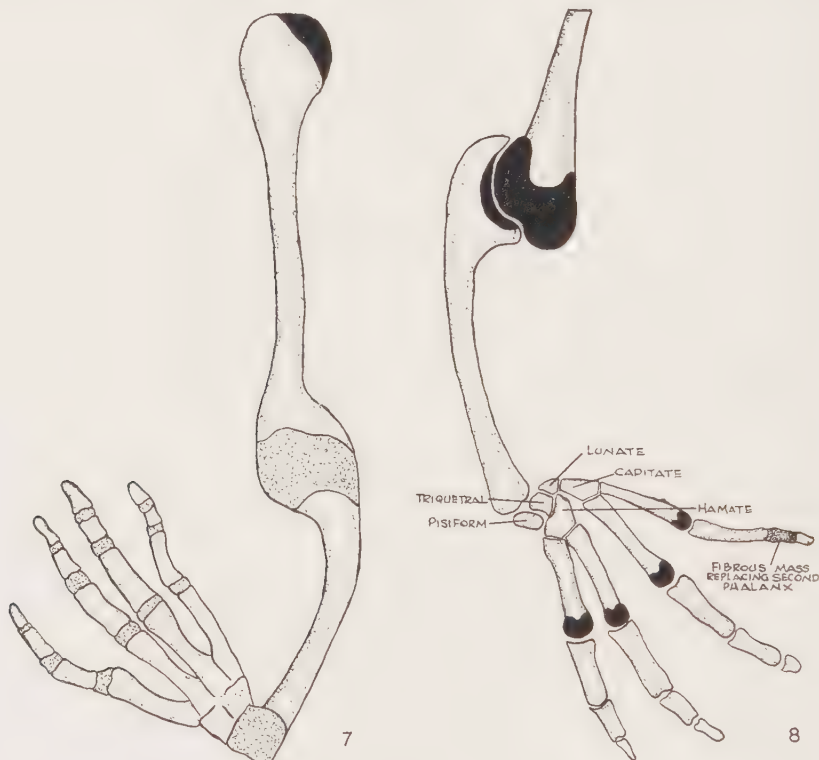


Fig. 7 Drawing of the bones and joints.

Fig. 8 Drawing of the skeleton of the forearm and hand.

the head of the bone is well developed and there is a small styloid process.

Of the carpal bones there are three on the proximal row—semilunar triquetral and pisiform—and two in the distal row—capitate and hamate. The scaphoid, trapezium, and trapezoid bones are missing. The pisiform is unusually large.

The three bones of the proximal row all articulate with the distal end of the anterior surface of the ulna. The semilunar articulates distally with the capitate and the hamate and medially with the triquetral. The triquetral articulates laterally with the semilunar, anteriorly with the pisiform, and distally with the hamate. The capitate articulates with the semilunar proximally and the hamate medially. The hamate articulates with the lunate and triquetral proximally and laterally with the capitate.

The index and middle metacarpals articulate with the capitate and the ring and little fingers with the hamate. The bases of all four metacarpals articulate with one another. The three lateral digits have each three normal phalanges. The index finger, however, while possessing a normal proximal and distal phalanx has no second phalanx. This bone has not chondrified and is represented by a mass of fibrous tissue. This suppression of a phalanx in the index finger is perhaps analogous to that which occurs normally in the thumb.

#### *Vessels and nerves*

The subcutaneous veins exhibit the usual arrangement.

The ulnar and median nerves are normal but the median supplies the muscles in the anterior compartment of the arm owing to the absence of the musculo-cutaneous nerve. The radial nerve ends abruptly just above the lateral epicondyle. The index, middle and the radial side of the ring finger receive their cutaneous supply from the median nerve, the ulnar supplying the little finger and the ulnar side of the ring finger (fig. 3).

The brachial artery does not divide into radial and ulnar at the elbow, the radial artery being absent. The ulnar artery gives off a large branch which passes down the forearm between the flexor digitorum profundus and pronator teres. This probably corresponds to the anterior interosseous artery. There is no *arteria mediana*.

The association of cervical ribs with congenital clubhand has been described in a number of cases (Funston, '32). No

cervical rib is present in this case but the first rib of the affected side, while having the usual muscles attached to it, is fused with the second rib from the position of the angle forward to the sterno-costal junction.

#### DISCUSSION

##### *Comparison of the findings with those in previously recorded cases*

The fusion of the biceps with the brachialis is unusual and though suggested as a possibility by Kato has not been described. The slip from the deltoid to the triceps is commonly found in clubhand cases as is also the fusion of the coracobrachialis and biceps (short head). In this case in addition to that fusion there is also a slip from the resulting muscle mass inserted in the normal position of the coracobrachialis so that this muscle has to a certain extent achieved its individuality. There is in addition the further unusual feature that the composite muscle formed by the fusion of the coracobrachialis and both heads of the biceps also sends a slip to the lateral head of the triceps. This muscle itself as is usual in these cases is normal apart from these two accessory muscular slips from the deltoid and the fused biceps and coracobrachialis.

The absence of the brachioradialis and extensor carpi radialis longus and brevis is usual and in this case the first two muscles mentioned are absent but there is also the presence of a small muscle that might conceivably be the extensor carpi radialis brevis. The extensor digitorum communis is usually inserted by three tendons into the third, fourth and fifth digits but in this case there are only two tendons running to the fourth and fifth fingers. The fusion of the extensor minimi digiti with the extensor digitorum communis is a usual occurrence.

The extensor indicis proprius is usually absent but if present has an abnormal insertion. In this case the muscle is present and its abnormal insertion is into the middle finger like an extensor tendon.

The palmaris longus is usually absent or may be fused with the flexor digitorum sublimis but in this case it is well developed and has an atypical insertion. The flexor digitorum sublimis and profundus muscles when present and not fused usually have three tendons each, running to the three medial digits, those to the index being absent. In this case there is a further variation in that the sublimis has only two tendons inserted into the middle and ring fingers while the profundus has only one tendon passing to the little finger.

The flexor pollicis longus tendon is almost always absent but here it is present and inserted into the base of the first metacarpal bone on the radial side. The pronator quadratus like the supinator is usually absent but may be inserted into the radial side of the carpus. This state of affairs is present here.

The insertion of the pronator teres into the ulna corresponds with the findings in several other cases and the thin tendinous slip extending down to the lower end of the ulna indicates a tendency for the muscle to be inserted into the radial side of the carpus as has been described in the literature.

The flexor carpi ulnaris is usually normal or only exceptionally absent but may have abnormal insertions; but, though the muscle here is normally inserted into the pisiform bone the extension round the head of the ulna to fuse with the extensor carpi ulnaris is most unusual.

The variations found in the blood and nerve supply to the limb are those that usually present themselves when the radius and thumb are absent.

*Aetiological theories reviewed with reference to the present case*

The theory of mechanical outside pressure seems a most unlikely explanation. The deformity as will be seen from the comparison of this with previously recorded cases presents a certain uniformity. Furthermore the total absence of a bone like the radius must have resulted from some disturbance



acting from at least the fifth week of embryonic life when the embryo would be so small that it is almost impossible to conceive of extraneous pressure that could affect such a localized area. This theory may apply to the so-called secondary congenital cases where the forearm bones are present though deformed.

The most acceptable theory is one of inherent cell defect or failure of the organizer responsible for the development of the limb occurring very early in embryonic life and resulting in suppression of certain portions of the embryo. The actual cause of this defect is not known. It has been attributed by Honel to rickets and by Peterson, Secord and Cluff to syphilis, while McCurdy attributes the condition to emotional upsets or mental diseases occurring in the mother during pregnancy. Virchow attributes the upset to some inflammatory process which has disturbed the development of the growing organism. Two factors which tend to support this theory of cellular defect are the frequent presence of other congenital defects in these cases and the degree of uniformity present as regards structures absent and modifications of these present.

Furthermore it has been demonstrated (Bagg, '29) that injury to the germinal cells can be produced by exposure to x-radiation and the descendants of the mice so treated exhibited various deformities of the feet which were inherited by their descendants for nineteen generations. This is the only recorded successful attempt to produce congenital defects experimentally and demonstrates that injury to the gametes before fusion may produce congenital defects in the resulting foetus. Whether the defect must be initiated in the gamete stage or whether injury of an unknown nature to the fertilized ovum or early embryo may also produce a congenital deformity is at present unknown.

The theory of nerve defect producing muscle defect is untenable as the musculocutaneous nerve is absent, yet the muscles of the anterior compartment of the arm supplied by it normally are here supplied by the median nerve and the muscles are well developed and exhibit some differentiation.

The theory that vascular defect causes the failure of differentiation and development of certain structures can be applied to this case with regard to the absence of the radial artery. The absence of the radius is in itself against this theory as here there is a large anterior interosseous artery which normally supplies the radius.

Of the muscles which normally receive blood supply from the radial artery the brachioradialis and thenar muscles are the only ones absent in this case though many of the others are abnormal. In the light of this isolated specimen it is therefore doubtful whether vascular defect is the primary cause of the abnormality.

An attempt was also made to explain the condition on the basis of a segmental defect of certain myotomes and their nerves. This supposition did not at all fit the condition found.

A study of the development and evolution of the skeleton of the upper extremity tends to show that the structures suppressed originate from the same embryological source. From the investigations of Götte and Strasser on Triton larvae and Wiedersheim on Proteus larvae it has been concluded by Goldmann that at some time during its development the carpus consists of three parallel rays of tissue which subsequently split into the individual carpal bones. From these rays there arise the digits. The radial ray gives rise to the thumb, the middle ray to the index finger, while the ulnar ray to the third, fourth and fifth fingers. The middle ray may be involved in abnormalities of either the radial or the ulnar rays. In the present case the radial ray has been suppressed so that the radius, trapezium, first metacarpal and the phalanges of the thumb are missing. The absence of the trapezoid which belongs to the middle ray and also the absence of the second phalanx of the index finger is an indication of the abnormality in the radial ray affecting the middle ray. This present specimen therefore fits in with the theory of the development of the hand as enunciated by Goldmann and supported by Kanavel ('32) in his review of congenital defects of the hand.

An explanation of radial clubhand based on the archipterygeal theory of Gegenbaur has been evolved. This postulates that the suppression of the first accessory ray has been the cause of the radial clubhand but as the archipterygeal theory has now been discarded and the carpus of the terrestrial creatures is claimed to have been evolved from the basal portion of the fin of one of the crossopterygian fish there is no point in pursuing this explanation further.

There were found in this case as in others previously reported shortened contracted muscles and joints with limited mobility or immobile. This fixation or limitation of mobility of the joints is due probably to the abnormal length and insertions of the muscles so that the development of normal joint cavities becomes unnecessary. Thus at the wrist joint, where the hand is acutely flexed toward the radial side and fixed in that position by the abnormal insertion of the pronator quadratus into the dorsum of the carpus which would tend to limit or prevent flexion and extension at that joint, the joint cavity has not been formed and the bones are firmly united by masses of dense connective tissue. Perhaps muscular defects round a joint prevent the normal absorption of the mesenchymatous mass filling the joint cavity. Thus in the elbow joint partial absorption of the mesenchyme has occurred but the presence of a ligament within the joint cavity dividing it into two subsidiary cavities shows that this process of absorption has been incomplete. On the other hand, the presence of muscles exerting abnormal tension on the bones forming the joint and tending to dislocate the joint may encourage the fibrosis of the mesenchymatous mass filling the joint cavity in order to ensure stability. A study of this case which exhibits a certain similarity to previously described cases, indicates that in view of the degree of muscular defect and abnormality and the presence of faulty joints, it is not to be hoped that more than a cosmetic result could be achieved by surgical treatment.

SUMMARY

1. Anatomical details of a case of congenital clubhand are described.

2. The findings are compared with those in previously recorded cases and a certain degree of similarity noted in all cases of congenital absence of radius.

3. Aetiological theories are reviewed and conclusion drawn that some inherent cell defect in the embryo is the probable cause of this condition.

4. The suggestion is advanced that muscular abnormality and defect lead to faulty joint development.

My thanks are due to Professor Low for placing the specimen at my disposal and for his helpful encouragement during the course of the investigation. I am indebted to Dr. Middleton Cannon for the radiograms of the limb.

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# VASCULAR SUPPLY OF THE HUMAN KIDNEY BASED UPON DISSECTION AND STUDY OF CORROSION PREPARATIONS

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ONE FIGURE

## INTRODUCTION

The present study is an outgrowth of an extensive investigation begun 6 years ago, dealing with anatomical corrosion preparations.<sup>1</sup> In the course of this work, such casts of different organs—lungs, pancreas, heart, liver, spleen, kidneys, etc.—were made and a number of interesting facts revealed which it seems are new or little known. Particularly in renal anatomy, conceptions are held which demand a revision and which will receive attention in the following report based upon the careful dissection and study of corrosion preparations of fifty-four human kidneys. In response to numerous requests, the author wishes also to describe the method which proved most satisfactory in the production of full-bodied, non-shrinking celloidin casts.

## HISTORY OF KIDNEY CORROSION

The history of kidney corrosion illustrates in large measure the course of development of the corrosion methods. Hosts of investigators have injected the kidney, but only those whose work has added some outstanding improvement in injection technique will be considered.

<sup>1</sup>The writer began his work of preparing corrosion specimens in 1931 under the direction of Prof. Otto F. Kampmeier, of the Department of Anatomy, of the University of Illinois.

The Greeks traced the course of blood vessels by inserting flexible sticks in them but Galen was probably the first to inject vessels by forcing air into them. Eustachius relates in his work, 'De Renum Structura,' that he injected water into the renal artery and vein and found it returning by way of the ureter; later he also demonstrated the converse. From these experiments he drew the erroneous assumption of a direct connection between the blood vessels and the uriniferous tubules—an idea accepted and taught until the time of Bowman (1848). Bellini (1665) injected the renal artery and vein through quills fastened to bladders of colored liquid, and a little later Malpighi, using ink in the same manner, saw glomeruli on surfaces made by cutting the kidney.

An important addition to injection technique was made by Robert Boyle (1668) who employed a fluid mass of plaster of Paris and gelatin which later hardened and left a cast of the renal artery and its larger subdivisions. By means of a syringe Ruysch (1727), filled both the renal artery and renal vein with molten wax. Both this mass and method have been used down to the present time with good results.

In 1882, Schiefferdecker described an injection mass consisting of celloidin dissolved in ether and colored by asphaltum which gave most gratifying results. His preparations of the kidney demonstrated both the finer and coarser circulation clearly and secured exact duplication of structure. Hochstetter (1888) added kaolin and Flint ('00) Prussian blue to prevent shrinkage. The next important contribution came from Krassuskaja ('04), who used a solution of photoxylin and camphor in acetone colored by cinnabar, Berlin blue, or chrome yellow. Both Huber and MacCallum quote these three investigators in their papers. A few years later, Huber suggested the use of old x-ray films and gave as the minimum effective dilution 3 parts of films to 100 of acetone.

## MATERIAL, METHOD, TECHNIQUE

Kidneys from persons varying in age from 2 to 70 years were secured from the necropsy table 12 to 24 hours after death. In addition, kidneys from pig, sheep, dog, and cat were injected and studied for comparison. Interesting points were noted in these animals which will be dealt with in a later publication. Immediately upon obtaining the kidney the vascular channels were washed out by fastening a flanged glass cannula into the renal artery and attaching to a pressure apparatus (fig. 1). Warm saline, at a pressure of 100 to 120 mm. of mercury, was used. A period of 15 to 30 minutes usually elapsed before the outflow from the vein returned clear. Then to prevent leakage of the chosen injection mass from any tiny perforating capsular branches during later injection, the kidney was submerged in water, injected with air, and the bubbling points tied off with waxed thread.

Five parts of washed, dried x-ray films per 100 parts of acetone was the routine mass used for injection. Rubber was added to the celloidin mass in an effort to temper and make it more tough, translucent, and non-shrinking; but as yet it is impossible to say if anything has been gained by the addition. The method of injection is simple and direct. An air pressure of 120 mm. of mercury—normal blood pressure—is maintained behind the injection mass by means of a constant pressure bottle and manometer. The acetone quickly combines with the water in which the kidney is submerged, causing the celloidin to precipitate out of solution in the channels that are being injected. As it is precipitating more mass is forced in by the continual pressure so that the cast solidifies from without inward producing an exact replica of the channels. In this manner kidneys were injected for 72 hours, afterward removed and kept under water for another 24 hours. The kidney substance was then macerated and removed with 10% hydrochloric acid. The dried casts were painted with Duco.

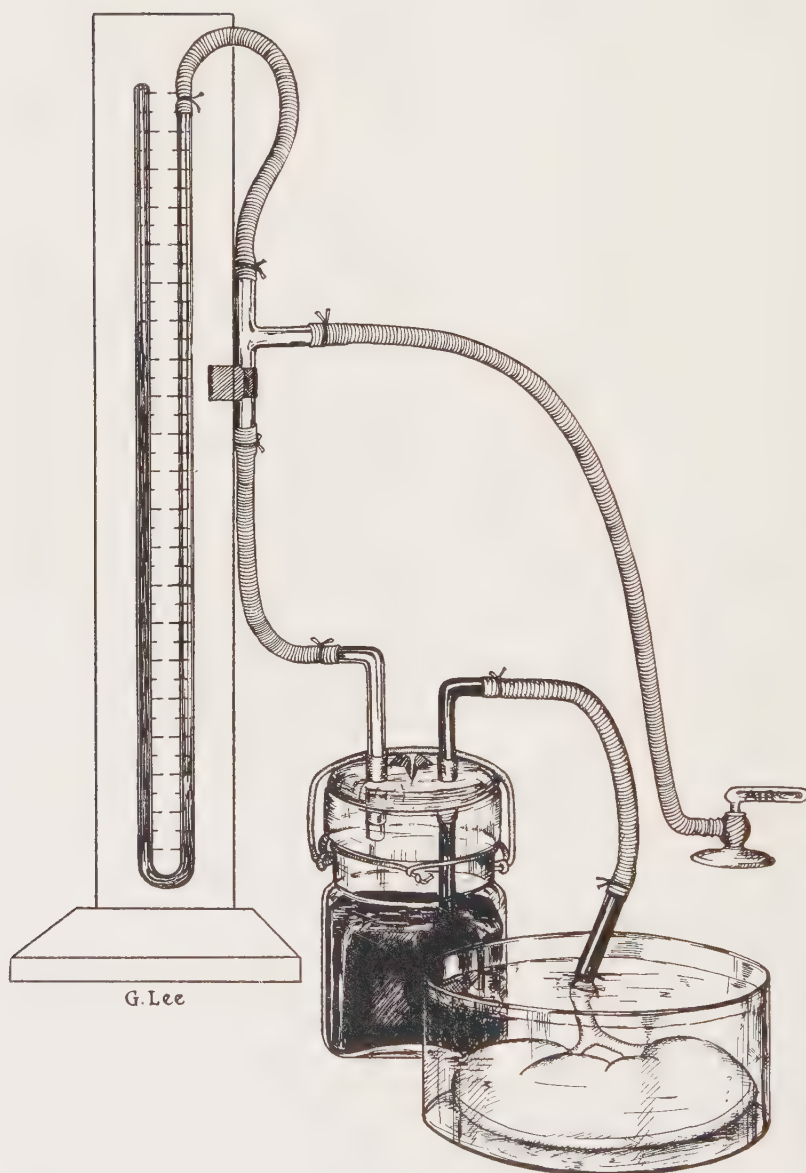


Figure 1

## OBSERVATIONS AND CONCLUSIONS

*Branching of renal artery in relation to hilum and pelvis*

Investigators for many years have attempted to discover and describe general patterns of division of the renal artery at the hilum of the kidney. Hyrtl, circa 1882, describes the kidney pelvis as completely separating the first branches of the renal artery into an anterior and a posterior system. Brödel ('01) gives support to Hyrtl's view and carries the separation farther laterally out into the pyramids and kidney cortex and describes an avascular zone in the latter vicinity. Kelly ('02) recognizes a diffuse division of the renal artery at the hilum. From a purely anatomical investigation, Albarron and Papin ('08) describes seven types of division of the renal artery at the hilum. Kuprijanoff ('24) divides his casts into groups also.

In studying the course and distribution of the larger vessels of the kidney the author used the term, first branches of the renal artery, to delimit only those first divisions which lie in anterior, posterior, superior and inferior relations to the kidney pelvis. They might just as well have been called interlobar arteries but the term interlobar was used here to describe vessels which were the continuation of these first branches as they entered the kidney substance, from the peripelvic fat, in relation to any major calyx or renal column of Bertini.

Corrosion preparations of fifty-four human kidneys were carefully studied and dissected. In forty-four specimens the arteries and pelvises were injected. In ten others triple injections were made consisting of the arterial tree, the venous tree, and the kidney pelvis. On the basis of dissection and study of all these corrosion specimens, it seemed a futile and fruitless task to attempt to describe any general pattern or patterns in the branching of the renal artery as it enters the renal sinus. The writer's casts revealed the greatest variety of divisions. The renal artery divided at the kidney hilum into three to seven first branches, all of which were found in



varying relation to the pelvis in the renal sinus. In each of the casts the greatest number, two to five, of first branches of the renal artery were found anterior to the pelvis. The most superior and the most inferior of these first branches as they course, respectively, superiorly to the upper pole and inferiorly to the lower pole come into a different relationship to the kidney pelvis as they leave the anterior plane. This observation will be amplified later in this paper.

In all casts the most superior of these first branches turned, at an angle varying from  $30^{\circ}$  to  $90^{\circ}$ , toward the most superior portion of the kidney pelvis. In no cast was an aortic polar vessel seen coursing to the region of the superior pole. In this same region no vessel appeared originating from the renal artery medial to the hilum and outside the kidney sinus which might be called a renal polar vessel.

The most inferior of the first branches of the renal artery, in all casts, turned toward the most inferior portion of the kidney pelvis at an angle varying from  $30^{\circ}$  to  $120^{\circ}$ . No aortic polar vessel was encountered here in any specimen. In three casts out of the fifty-four the most inferior of the anterior first branches originated from the renal artery medial to the kidney sinus and hilum and pierced the kidney substance inferior to the hilum. The author believes them to be clear-cut examples of the so-called renal polar vessels. Two were in left kidneys and one in a right one. All three of these, after dissection, were found to supply a much wider area than just the inferior pole of the kidney. The observation is more clearly described by saying that the vessel nourished the entire inferior third of the kidney. All three renal polar vessels coursed inferiorly, at an angle of  $45^{\circ}$  to  $90^{\circ}$ , anterior to and resting upon the junction of the pelvis and ureter on a plane posterior to the renal vein at the hilum.

One to three first branches of the renal artery were found posterior to the kidney pelvis in the renal sinus. No anastomosing of any of these first branches of the renal artery were seen.

*Course and relations of interlobar arteries*

Textbooks showed diagrams of arteries running to and arching around bases of pyramids in the corticomedullary zone. They were drawn with convex aspects facing laterally and parallel to the lateral convex border of the kidney and called arciform arteries. Braus said emphatically in 1923, "Der Name Arteriae arciformes für sie, welcher allgemein gebräuchlich ist, ist direkt falsch." Casts on which the present study was based confirm this judgment, and support the following description of the interlobar arteries: as they left the periphery of the renal pelvis they divided dichotomously at successive intervals and coursed toward any surface or border of the kidney with their concavity, much more frequently than their convexity, toward the lateral convex border of the organ.

In the French literature the term arciform was rarely used. A search for the originator of the term has been fruitless. Henle used the term when describing the veins of the kidney. The triple injection casts, as they were dissected, showed many veins arching around the pyramids to reach the anterior aspect of the kidney but the arteries were not observed to do so in any of the casts. So the question arose whether the term arciform, which adequately described the veins, had not been erroneously carried over to describe arteries; since we have been taught that veins followed fairly closely the arterial pattern. In every specimen of the venous tree all of the veins from the posterior surface coursed anteriorly to drain into the renal vein which at the hilum was found anterior to the artery and pelvis. In the case of the venous circulation, arciform was an appropriate descriptive term to apply but not in the case of the arterial tree. The veins arched anteriorly in relation to the anterior row of calyces but the arteries did not. More information concerning the venous drainage will be offered in a later communication when the course and branching of the interlobular arteries will also be considered.

A striking observation appeared in the crossing over of interlobar arteries from anterior to posterior and vice versa.

In the region of the superior pole the interlobar vessel divided dichotomously, usually, into three branches. An anterior one coursed anteriorly to the major and minor calyces and supplied pyramids and cortex of the anterosuperior region of the kidney. The pyramids and cortex of the posterosuperior region were supplied by a superior branch which was usually found lying above the uppermost major and minor calyces. On its course it crossed posteriorly over these latter structures. Finally, a posterior branch crossed more directly posteriorly than the superior branch, and supplied pyramids and cortex in the posterior region of the upper third of the kidney. Here we have seen two interlobar vessels which crossed posteriorly from the anterior surface of the kidney pelvis. One of them coursed posterosuperiorly and the other more directly posteriorly. In all casts the superior pole and in many casts the entire upper third of the kidney were supplied by the most superior of the anterior first branches, due to this crossing.

The interlobar vessels in the middle third of the kidney were carefully dissected and studied. In thirty-five casts, or 64%, from two to six anterior interlobar arteries crossed posteriorly just lateral to the pelvis to supply pyramids and cortex on the posterior aspect. An attempt was made to count all interlobar vessels to determine the number which did not cross. Their repeated dichotomous branching soon made this determination so difficult that it was abandoned. In three casts, two to three branches of posterior interlobar vessels crossed anteriorly and supplied pyramids and cortex of the middle third of the kidney.

In the region of the inferior pole of the kidney essentially the same picture prevailed as that already described at the superior pole. In all of the casts both the superior and inferior poles, and very frequently the entire upper or lower third of the kidney, received blood through anterior first branches of the renal artery. This was due to the crossing of the interlobar vessels in these regions. No posterior first branches were found to supply the poles of the kidney. In

some of the casts the upper and lower thirds, however, received interlobar branches from these first posterior divisions.

In 64% of this series of corrosion preparations in the human there was no so-called 'avascular zone' in the middle third of the kidney. All the casts showed anterior interlobar vessels crossing posteriorly at both poles which ruled out 'avascular zones' there. When the triple injected casts were studied the 'avascular zone' was even less apparent. In these casts, in addition to the interlobar arteries, all the venous arches crossed anteriorly through it.

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## SOME OBSERVATIONS ON THE GRAAFIAN FOLLICLES IN AN ADULT HUMAN OVARY

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ONE TEXT FIGURE AND TWO PLATES (FOUR FIGURES)

Numerous articles on human polyovular follicles and polynuclear ova have appeared since the reports of Nagel (1888), Stöckel (1898), and Rabl (1899). The reader is referred to the comprehensive review of this entire subject by Hartman ('26). A most unusual case of polyovular follicles in the human was reported by Arnold ('12), who found a total of eighty-eight larger follicles containing more than one oocyte. Although the literature is voluminous, there still are stages in the growth and degeneration of human ovarian follicles which have not been fully described.

The occasion for this report was offered by what seemed to be unusual human material. A comprehensive description of the fate of graafian follicles has been given by Shaw ('25), Schröder ('30), Allen et al. ('30) and Garde ('30). The material here presented may add further information on the growth and atresia in the human follicle.

The ovary here described, was a surgical specimen obtained from a local hospital. Unfortunately the only data obtainable was that the tube and ovary had been removed from a woman 25 years old. The ovary was cut in halves longitudinally and fixed for a number of days in Bouin's fluid. It was embedded in paraffin, sectioned at 10  $\mu$ , and stained in Delafield's hematoxylin and eosin. No attempt was made to obtain a perfect series, since the material was to be used for routine class work.

On the slide the sections measured 22 by 8 mm. and showed many vesicular follicles (fig. 1). Microscopic examination of all available sections revealed two vesicular monovular follicles at A and B (fig. 1). These follicles had an average diameter of 2 mm. One (B) contained an intact cumulus oophorus with a normal ovum. This ovum had a round vesicular nucleus centrally located, and a definite nucleolus. The cumulus oophorus was near the outer edge of the follicle and contained numerous mitotic figures. The other follicle (A) showed signs of degeneration. The cumulus oophorus and the stratum granulosum were beginning to fragment and the ovum stained faintly. In the other pole of the ovary



Fig. 1 A diagram of a longitudinal section through the ovary to show the location of the follicles described. A and B, monovular follicles; C, biovular follicle; D, follicle with four oocytes.

was a larger follicle (C) which measured 4 by 2 mm. and contained two ova. Its cumulus oophorus and stratum granulosum were partially detached from the wall of the vesicle (figs. 1 and 2). The cortex over this follicle was rather thick. Both ova appeared normal and measured 55 to 60  $\mu$  in diameter within the zona pellucida. The nuclei and nucleoli were distinct and the nuclear membranes were intact. One nucleus was vesicular while the other was chromatic (fig. 2). The cortex contained other follicles at various stages of development, but relatively few primary follicles. There were numerous corpora of atresia in the various stages previously described by Shaw ('25) and Allen, et al. ('30). No definite

corpora lutea were found. In the cortex just lateral to the mesovarium at D (fig. 1) was a pyramid-shaped vesicle which had a thick layer of ovarian stroma over it. Its greatest diameters were 2.5 by 1 mm., and it was wedged between a corpus albicans and another vesicular follicle (fig. 3). The wall of this vesicle was thick and composed of theca intima cells, a glassy membrane with flattened cells lining the cavity. No stratum granulosum was present, and the entire structure of the wall was similar to the atretic follicle shown in figure 5 by Shaw ('25). Free within the vesicle was a mass of pale cells, loose granulosum cells which contained four oocytes all in one zona pellucida, and what appeared to be a vitelline membrane (figs. 4 and 5). The greatest diameters within the zona pellucida were 90 by 110  $\mu$ . This zona pellucida with its oocytes was found in nine sections (90  $\mu$ ) which were arranged in serial order, and probably represented the entire structure. In one of the sections (fig. 4) was a cell within the zona pellucida which was interpreted to be a macrophage. Many other sections through this follicle were studied and the continuity of the vesicle ascertained. These oocytes were polynuclear and their diameters varied from 30 to 40  $\mu$  (figs. 4 and 5). Each oocyte had one to three round or vesicular nuclei which varied in size. The nuclei each had one to three eccentrically placed nucleoli. At N in figure 5 were found two small bodies that stained and appeared like extruded nucleoli. No indications of mitosis were observed. The writer is led to believe that segmentation had occurred amitotically. The available literature appears not to have any figures showing such a process in the human. In the textbook of histology by Maximow and Bloom is found this statement concerning atresia of vesicular follicles: "Amitotic fragmentation of the nucleus and division of the protoplasm also occur. These changes result in the appearance in the interior of the zona pellucida of several cell bodies of varying size and with more or less distinct nuclei." A similar process is mentioned by Schottländer (1893) and Mossman ('37).

## SUMMARY

This paper presents some information on the growth and degeneration of follicles in an adult human ovary.

Two vesicular monovular, one large biovular, and an unusual vesicular follicle which probably represents a type of atresia are described.

It appears that many follicles had become large and vesicular and then degenerated, since no definite corpora lutea were found.

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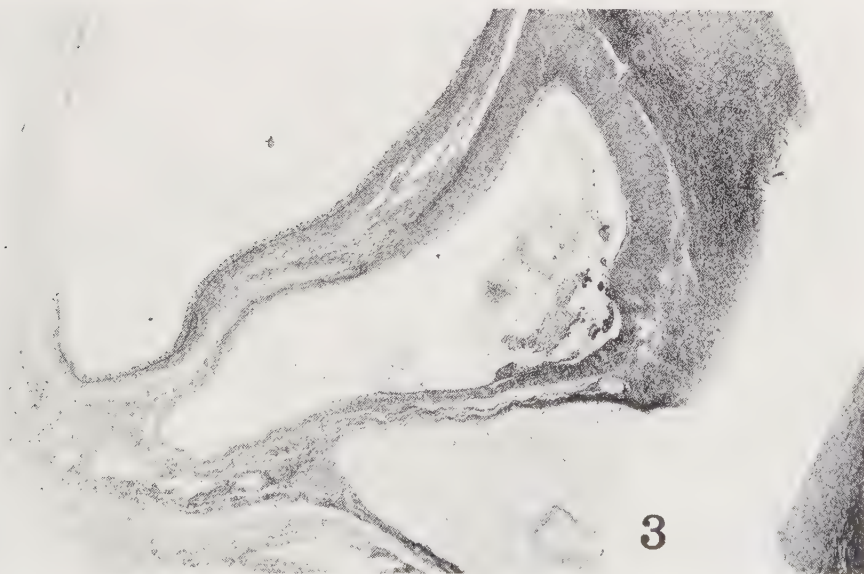
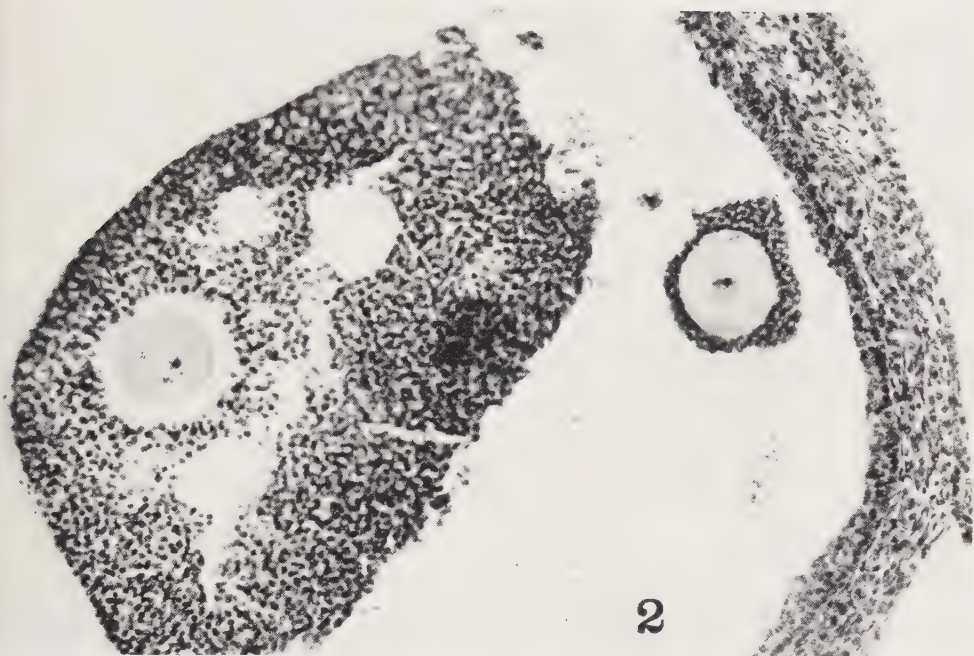
## PLATES



## PLATE 1

### EXPLANATION OF FIGURES

- 2 Photomicrograph of the follicle at C, which contained two oocytes.  $\times 200$ .
- 3 Photomicrograph of the follicle at D, which contained four oocytes within one zona pellucida. Note how it is wedged between another vesicular follicle and a corpus albicans.  $\times 30$ .

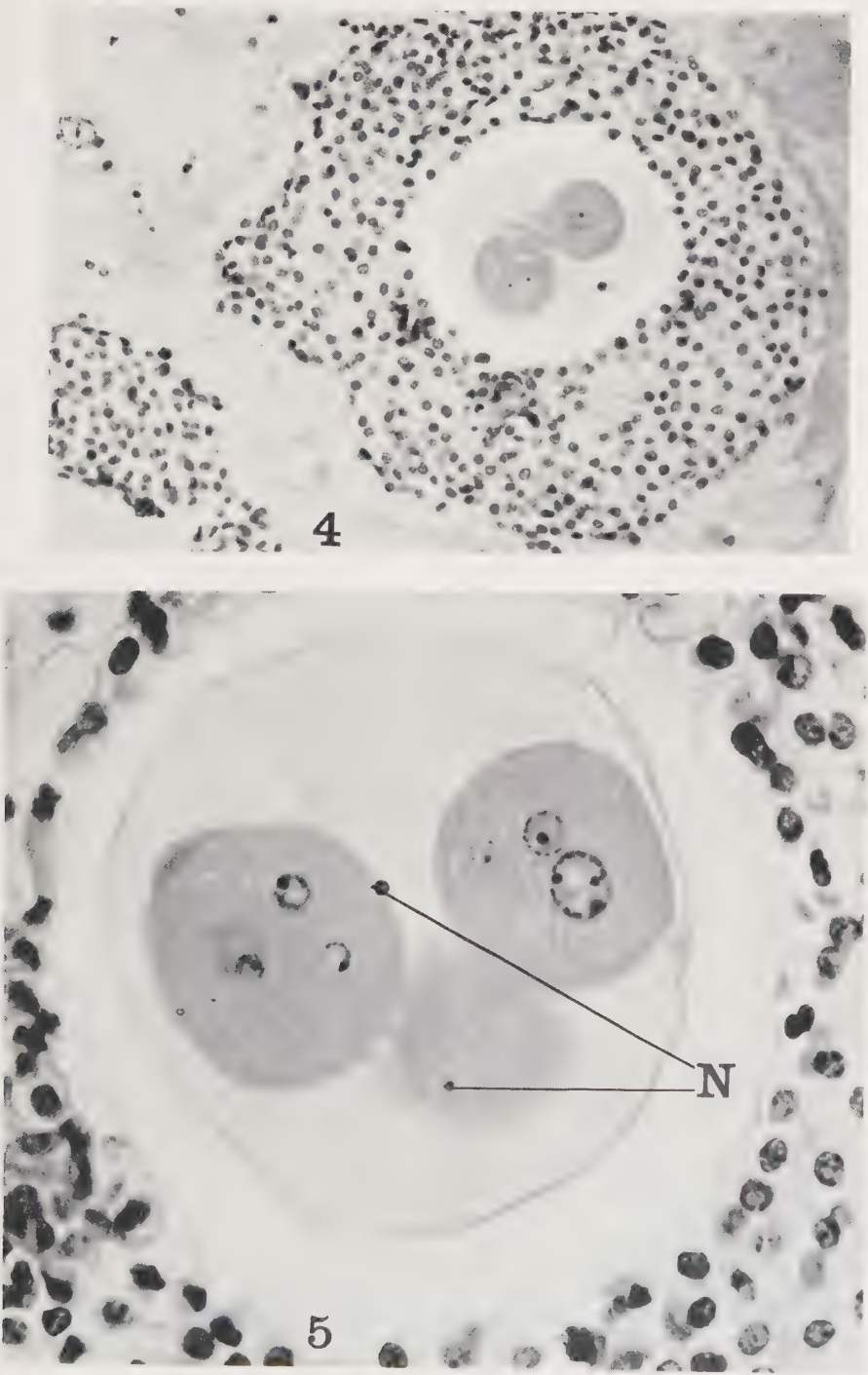


## PLATE 2

### EXPLANATION OF FIGURES

4 Photomicrograph through follicle (D) to show two oocytes, one binuclear, and a phagocyte within the zona pellucida.  $\times 250$ .

5 Photomicrograph to show three oocytes, all in the same zona pellucida. Note the polynuclear oocytes.  $\times 750$ .







# A MICROSCOPIC STUDY OF THE SURFACE ENAMEL OF HUMAN TEETH

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TWO PLATES (EIGHT FIGURES)

A survey of the available literature on human enamel prisms reveals only casual references to the surface ends of the prisms. Mummery ('24) has referred to surface enamel incidentally in his discussion of the so-called Nasmyth's membrane. The impressions made by the superficial ends of the prisms on this membrane have been described, and Chase ('26) in his study on the origin and structure of the membrane has given a full account of the impressions. Because of the neglect of this phase of enamel structure, it was considered that direct observations on the surface of the human tooth would disclose information helpful in evaluating the vast data upon dental caries. Aside from this, details on surface structure of the tooth are presented.

## MATERIAL AND METHODS

Fifty selected teeth, extracted in routine office procedure, form the basis of this report. The reasons for extraction, and the number in each category, are as follows: a) loose because of alveolar absorption, twenty two; b) unsuited for retention in the mouth because of mechanical reasons for restoration efficiency, eighteen; c) pulpal involvements, eight; d) unclassified, two. After extraction all teeth were preserved at once in 10% formalin. They were cleaned for study by the application of a disclosing solution (I + KI) and subsequent polishing with a dental polishing brush and ordinary tooth

paste; to insure cleanliness of the tooth surface this process was repeated. The presence of a delicate surface sheath or membrane (the so-called Nasmyth's membrane) on fully erupted teeth seems highly unlikely (Chase, '26). Such a membrane, if it exists, would certainly be completely removed in the process of polishing. The teeth were then re-immersed in the formalin solution from which they were removed for microscopic study. All observations were made with a Leitz Ultropak microscope, employing magnifications of from  $\times 30$  to  $\times 750$ .

#### OBSERVATIONS

Under low powers,  $\times 30$  to  $\times 100$ , no structural details of enamel rods are visible, but teeth show ripple marks (fig. 1). These marks traverse the crown of the tooth transversely in the incisal and cervical third. In the middle third they are usually obliterated because of masticatory abrasion. Such abrasion marks or scratches are present in all teeth, their direction usually parallel to the long axis of the tooth. In addition, a few show abrasion marks parallel to the occlusal surface. Some teeth present checks or cracks running in a direction parallel to the long axis of the tooth. However, these cracks do not open on the surface in the majority of cases, so that no additional mention need be made of their appearance.

At intermediate magnifications, the ripple marks reveal certain details. The contour of these ripple arcades is not a smooth wave, but rather a wave which reaches a more or less sharp crest with the crest break directed toward the neck of the tooth. It was found that the best detail of enamel rods is visible in the beginning of the ascending portion of the ripple (fig. 2). Good rod detail can also be seen on the edge of the crest, where the rods seem to jut out and form a fringe (fig. 3). The distance between crests varies from 30 to 50  $\mu$ ; the depth from 5 to 10  $\mu$ .

The best detail of enamel rod structure is obtained with a  $\times 50$  water-immersion objective and a  $\times 6$  or  $\times 15$  ocular.

With still higher powers the illumination becomes poor and observation of details is impossible. At the incisal edge few teeth present rod detail. Whenever rod structure is present, the rods are consistently broken up and the general appearance is almost structureless and non-reflective. As the observation of the tooth surface proceeds in a direction away from the incisal edges, the rod grouping can be seen clearly. No definite geometrical pattern can be detected although some evidences of pattern groupings can be observed. Such patterns always indicate extremely wide spirals of a sinistral or left handed character. Observations on the middle third of the tooth reveal, usually, an irregular mosaic of rod ends. On the mesial and distal portions of the crown the rods tend to form a more regular mosaic. In general the rods are closely packed with thread-like hyaline spaces about each rod end. There are, however, many small areas (figs. 4 and 7) measuring from 200 to 500  $\mu$  in diameter, where rod end detail is entirely lacking. These islands of almost structureless non-reflective enamel are punctuated with small groups of brightly outlined rods of a diamond or semilunar shape. This observation of the mosaic pattern of rod ends being broken up by areas devoid of rod structure is not confined to one type of tooth or to any particular surface or region, although it is more commonly found on the labial surface of incisor teeth. In general, the rods about such areas lack the semilunar appearance, being rather more angular or presenting a quadrilateral shape with some modifications. Usually, the rod mosaic is more homogeneous and regular as the neck or lower third of the enamel cap is reached.

The shape and size of the rod ends varies with the locality on the tooth where the observations are made. The most common rod end shape is more or less hexagonal or a distorted semilunar outline showing evidence of five or six sides (figs. 5 and 8). Some areas present an appearance similar to scales on a fish or a butterfly wing. The greatest variety of rod end shape is found in the middle third of the enamel cap.

There the scalloped appearance is the most frequent form encountered. In this region, where a bulge or small cusp (like the cingulum on the palatal surface of a cuspid) is formed, a considerable length of the rods is occasionally exposed. At times it is possible to follow rods for a distance equal to from five to ten times their width. These rods are usually wavy in appearance, making one-half or one full wave in their course (fig. 6). Most rods surrounding the non-reflective areas (described above) present a more or less diamond or four-sided shape. True hexagonal rod end forms are most commonly found on bicuspid and molars, and there most frequently in the incisal half of the enamel cap. As the enamel progresses toward the neck of the tooth semilunar shapes become more evident. All rod ends, no matter where located, seem to show a brilliant peripheral band and a non-brilliant internal zone. On slightly etching the surface of some of the teeth with 5% nitric acid for 2 to 3 minutes the rod matrix, corresponding to the bright periphery, seems to stand out, and the non-brilliant zones seem to have receded in position, indicating that the matrix is of higher organic content than the rod substance itself. Many rod ends are filled with brilliant spots; the unevenness of the refractibility of light by these rod ends is very striking. In groups of fifty to seventy-five rods, there are two or three dark rod ends and twenty-five to thirty rod ends more brilliantly outlined. Interspersed among these brilliant rod ends there is an occasional rod standing out as if internally illuminated. These ultra-brilliant rods seem to be arranged in small groups or clusters, and are estimated to be about  $15\mu$  above the average field level. The size of the rod ends varies in different locations. Toward the neck of the tooth their area progressively decreases, the brilliant peripheral band becomes thinner, and the non-brilliant zone becomes relatively larger. Rods of larger diameter are present about the incisal region, and also about the middle third of the tooth, corresponding to its greatest diameter. The average diameters of the rods, computed from measurements of fifty rods on each tooth examined

(2500 rods altogether) are: largest,  $6.8\mu$ ; medium,  $5.7\mu$ ; smallest,  $4.5\mu$ .

#### DISCUSSION

Observations on surface enamel reflect the structure of enamel prisms as already described by Smreker, ('05), Walkhoff ('03, '04, '30), and others who studied ground sections of enamel. It is well-known that enamel prisms while parallel to each other are not always perpendicular to the surface of the tooth. Lehner and Plenk in von Möllendorff's 'Handbuch der Mikroskopischen Anatomie des Menschen,' ('36), have illustrated schematically this feature. The tortuousness of enamel prism accounts, in part, for the presence of a greater exposure of rods on the surface. This study does not reveal the reason for differences in reflectibility between the periphery and centers of the enamel prisms or for the brilliant spots observed in some rods. Moreover, the significance of the non-reflective areas is rather speculative, although great importance might be attached to this observation as a hint that these areas may be incipient caries. If caries, as is generally believed, begins at the surface of the tooth, rod detail would be destroyed before actual liquefying destruction began. Another possibility would be the failure of rod formation, a form of microscopic hypoplasia.

#### SUMMARY

Enamel rod ends are visible over practically the entire surface of the polished human tooth. The most common rod end shape is more or less hexagonal, or a distorted semilunar outline showing evidence of five or six sides. Over the widest diameter of the tooth, and on small cuspal prominences of the palatal surfaces of incisors and canines, rods may be exposed and lie lengthwise for small distances. Islands of non-reflective enamel, showing no rod detail, are visible in many instances.



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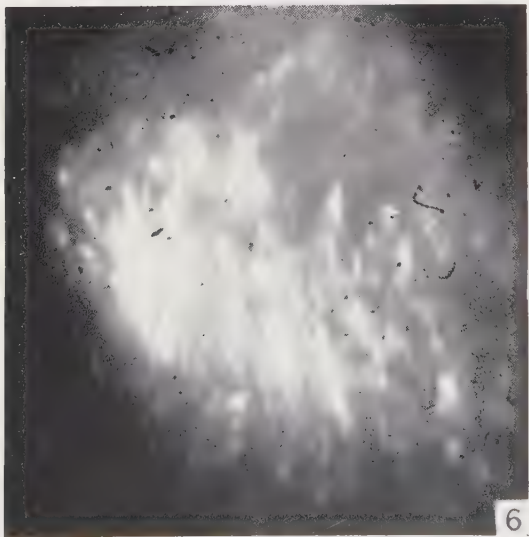
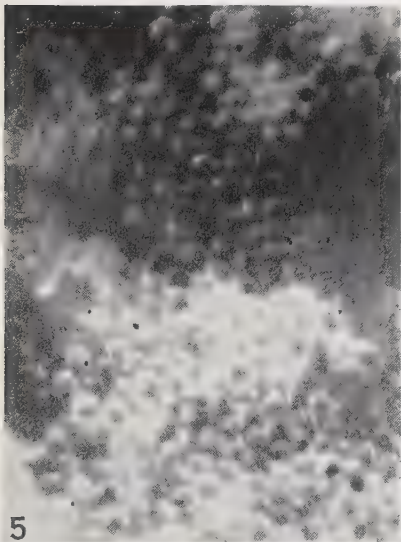
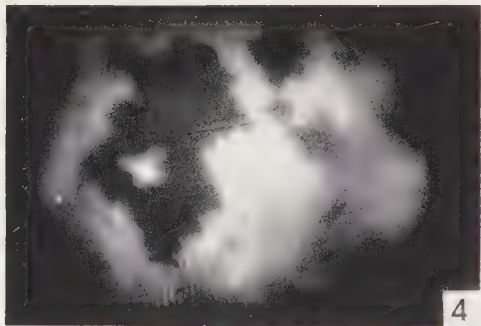
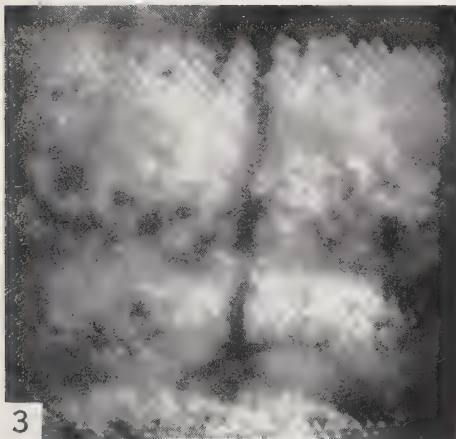
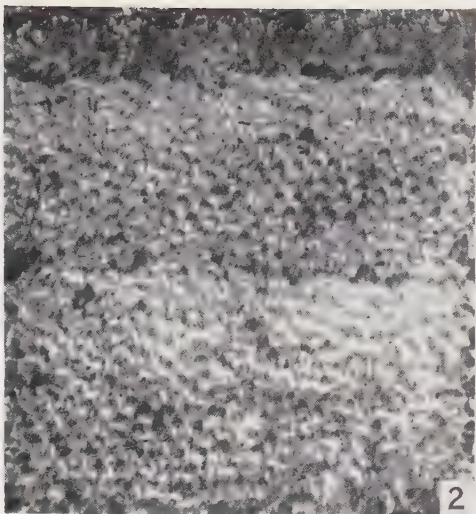
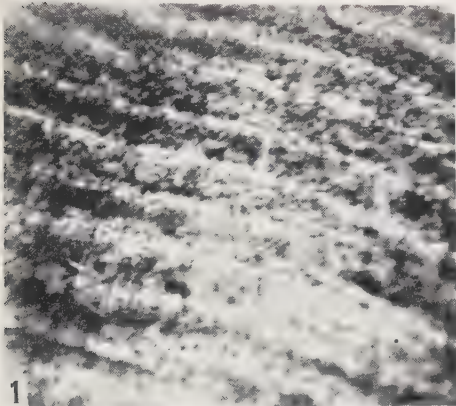
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## PLATES

## PLATE 1

### EXPLANATION OF FIGURES

- 1 Surface enamel showing growth arcades or ripple marks.  $\times 100$ .
- 2 Rod end mosaic on ascending portions of ripple. Observe distorted semi-lunar shapes.  $\times 700$  dry.
- 3 Rod mosaic at crest of ripple. Observe quadrilateral rod end shape.  $\times 450$  water immersion.
- 4 A typical island of structureless non-reflective enamel with highly reflective enamel surrounding it.  $\times 150$  dry.
- 5 Typical area of enamel showing variety of shapes of rod ends and variation in reflectibility.  $\times 750$  water immersion.
- 6 Partially exposed enamel rods.  $\times 450$  water immersion.



## PLATE 2

### EXPLANATION OF FIGURES

7 Arrangement of rods about border of non-reflective enamel. Photographed at 750 diameters and enlarged for reproduction, it represents a field of figure 4. Notice the rod ends are roughly hexagonal and pentagonal.

8 Enlargement of lower half of figure 5.







# STUDIES OF THE MECHANISM OF ELIMINATION OF EGG ALBUMEN AND ITS EFFECT ON THE ELIMINATION OF IRON BY THE RABBIT'S KIDNEY

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## TWO FIGURES

In a consideration of the mechanism of the elimination of egg albumen several questions present themselves. First, in what part of the nephron is the elimination localized? Second, is the elimination of this albumen a process of filtration or secretion? Third, does the passage of the albumen affect the cells through which it passes?

Many investigators (notably Adami, Schmidt, Miller, Fukuda, Oliver, Whipple and Gersh) have sought to answer these questions for the elimination of hemoglobin. Variouslly they conclude that hemoglobin may be eliminated through the glomerulus or through the proximal tubule or through both. By some it is considered to be a process of filtration, by others a process of secretion, by others a combination of both, and Gersh ('36) postulates a process of 'leaking' in addition. Among the few who consider at all the effect of the hemoglobin on the cells through which it passes, some find no effect and others conceive changes in the permeability of the capillary walls.

Bayliss, Kerridge and Russell ('33) in a series of experiments with various proteins injected into anaesthetized cats

<sup>1</sup> This is a brief report of a preliminary series of observations on the elimination of egg albumen and iron. The author wishes to express her gratitude to Dr. S. H. Bensley for her suggestions and continued interest in the work.

and rabbits and with perfusion of dog's kidney concluded that the glomerular membrane is normally permeable to some proteins, specifically those having a molecular weight of less than 70,000. Histological examination showed no evidence of renal damage and protein was seen in the capsular space in two experiments in which egg albumen (with a molecular weight between 33,000 and 35,000) was excreted in high concentration. Hemoglobin, with a molecular weight of 68,000, was found to be excreted only when its plasma concentration exceeded a certain level, and its concentration by the kidney was small.

In considering this group of experiments the question arises: Are the criteria for the exclusively glomerular elimination of these substances and for the absence of changes in the renal epithelium adequate? At present there are no histological methods by which foreign proteins may be demonstrated in cells (except for hemoglobin, in which case the color in the section is distinctive), so that the possibility of elimination of protein (except hemoglobin) by the proximal tubule cells cannot be determined in sections alone. The treatment of the tissue in order to preserve and demonstrate the protein at the site of its elimination is not always adequate for preserving cytological detail and for the demonstration of minute changes in structures which are as delicate and intimately related as the various components of the glomerular membrane and the cytological constituents of the tubule cells.

A number of investigators (notably Stieglitz, '21, and Holton and Bensley, '31) have shown that normally iron injected in the form of ferric ammonium citrate is eliminated by the proximal tubule cells and the glomerular spaces are uniformly free of iron. It had been noted by Bensley (personal communication) that in the rare instances when minute amounts of iron occurred in the glomerular space, precipitated protein was also present there. If iron injected in moderate amounts cannot go through the glomerular membrane but does in combination with albumen, in concentrations great enough to be detected microchemically, this would suggest a change in the permeability of the glomerular membrane.

Gersh ('36) indicates that this is so. In discussing the mechanism of the elimination of hemoglobin he refers, in passing, to one experiment "in which iron ammonium citrate was introduced intravenously in a rabbit some time after an injection of hemoglobin. Iron was definitely found in the glomerular space although it has never been demonstrated there in normal rabbits injected with iron alone."

One such experiment, however, cannot indicate whether the glomerular membrane was made permeable to iron by the presence of hemoglobin, or by the combination of hemoglobin and iron, for it gives us no data showing whether the glomerular passage of hemoglobin causes or follows changes in the permeability of the glomerular membrane.

The following experiments which were a part of a study of experimental albumenuria in rabbits may indicate a method of approach for the solution of this complex (and confused) problem.

The experiments were made on five groups of animals:

1. Twenty-one male rabbits injected intravenously with 5 cc. each of a 50% solution of egg white in water, filtered through filter paper.

2. Eleven male rabbits injected with 5 cc. each of 50% aqueous egg white a second time, 2 to 8 days after the first injection.

3. Nine male rabbits injected with 10 cc. each of a 10% aqueous solution of ferric ammonium citrate (green scales).

4. Eight male rabbits each injected intravenously with
  - a) 5 cc. of 50% aqueous egg white, immediately followed by
  - b) 0.2 gm. per kilogram body weight of ferric ammonium citrate in 10 cc. of water.

5. Eight male rabbits injected with egg white and iron salt in the same manner as those in group 4 and sacrificed at appropriate intervals for histological studies.

The rabbits were kept in metabolism cages for several days preceding the experiments. The urine was collected and analyzed to determine as far as possible that it was normal. From catheter specimens of urine, collected after the intravenous



injection, elimination curves were plotted. The acetic acid test with heat was used for the detection and estimation of albumen, the values, checked against standard solutions, being recorded as trace, 1+, 2+, 3+ and 4+. The Prussian blue reaction developed with 1% HCl and 5% sodium ferrocyanide, freshly prepared, was used for the estimation of iron, the values, checked against standard solutions, being recorded as 1, 2, 3 and 4 plus. Because of irregularity in the collection of samples, volumes were corrected by the factor:

$$\frac{\text{Volume in cubic centimeters}}{\text{Number of minutes since last sample}} \times 10$$

which would be a corrected rate, read in tenths of a cubic centimeter per minute.

Pieces of kidney from the animals in group 5 were fixed 1) by absolute alcohol chilled to  $-62^{\circ}\text{C}.$ , 2) by boiling for 1 minute and transferring them to 85% alcohol, 3) by 95% alcohol at room temperature, and 4) by modified formalin-Zenker, i.e., 1 part neutral formalin to 9 parts Bensley's chrome-sublimate solution.

The chilled alcohol proved to be the best fixation for localizing the iron, which was visualized by the Prussian blue reaction (Stieglitz, '21). There was less fading after fixation in 95% alcohol, however. The study of iron elimination was made on  $20\mu$  sections. Sections of tissue fixed in formalin-Zenker,  $6\mu$  in thickness, stained with Mallory's connective tissue stain proved to be the most valuable for the detection of albumen and of cellular changes.

Before describing the results of these experiments the possible sources of errors, which must be taken into account in interpreting and evaluating the results, should be mentioned. Although the rabbits appeared to be normal before experimentation some of the animals died or developed diarrhea during the course of experimentation. This source of error was eliminated in the later experiments by obtaining a pure strain of healthy Swift's snuffle-free rabbits.

The catheter specimens could often be collected only at very irregular periods. Although these were corrected by a factor for comparison of rate, they cannot thereby express what was occurring at the time when the sample was collected. Obviously if a long interval elapses between specimens with a considerable change in the concentration of the injected substance, no evidence can be obtained as to when during that period the progress of change occurred.

At the time of these experiments only Merck's iron ammonium citrate (green scales) was available. This product, as Stieglitz ('21) points out, is toxic when injected intravenously and therefore the iron injection experiments had to be acute. Stieglitz found, however, no apparent changes in any tissues after the injection of as small quantities as were used in these experiments.

It is obvious that the tests used for determining the amounts of albumen or iron in the urine are only grossly quantitative. Doubtless the maximum plateau exhibited for iron elimination should be resolved into a rise and fall. But the intensity of the blue color attained at the 4+ reading is so great that no further increase can be detected at this concentration. No attempt was made to determine whether the albumen excreted was purely egg albumen or a mixture of it with proteins derived from blood or tissue destruction.

Since the elimination curves were made primarily for the purpose of determining the time intervals at which to sacrifice the animals in group 5, complete histological studies were not made on animals injected with albumen alone.

A study of the elimination curves (of which those in figures 1 and 2 are fairly representative) reveals the following results, which are summarized for convenience:

1. Following the first injection of albumen, this substance appeared in the urine within the first 20 minutes only three times, even though samples were collected within that interval in seventeen animals. In five animals the urine was negative for albumen for from 25 to 63 minutes.

2. Following the second injection of albumen, it did not appear in the urine in any case within the first 20 minutes, even though samples were collected within that interval in five animals. In five animals the urine was negative for albumen for from 30 to 91 minutes.

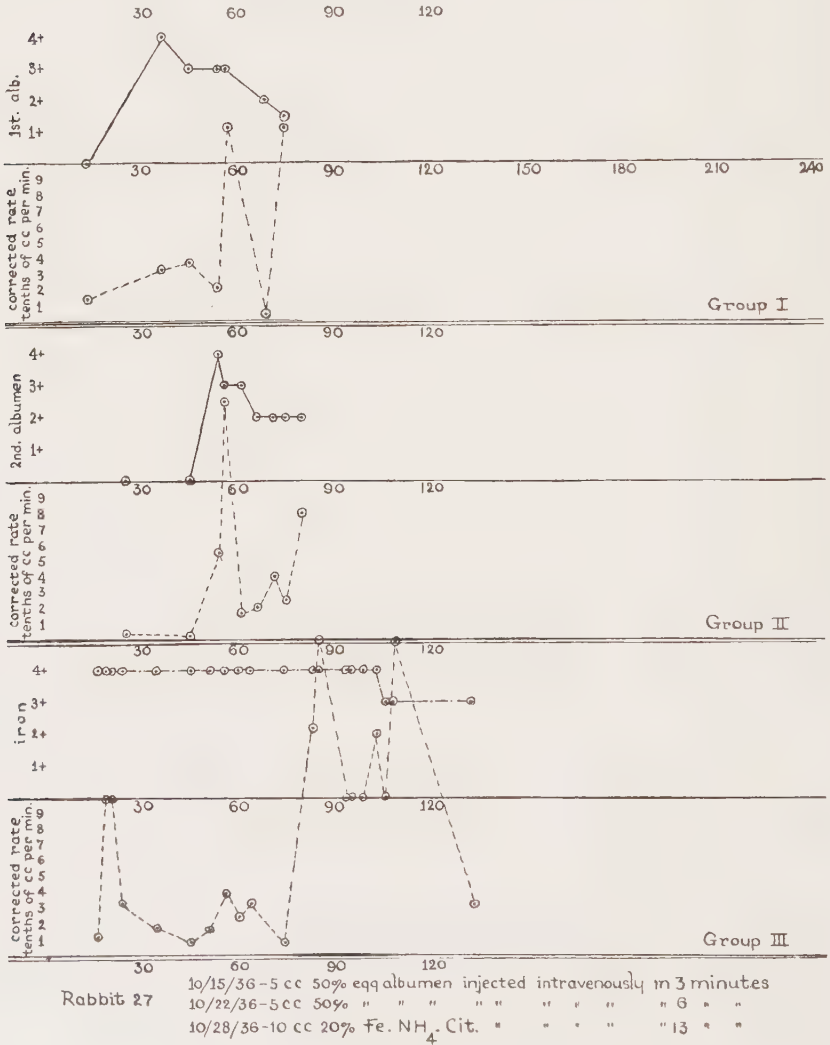


Figure 1

3. In both groups, when frequent samples could be collected, albumen elimination usually reached the maximum within 2 to 6 minutes after its first appearance or following a negative specimen.

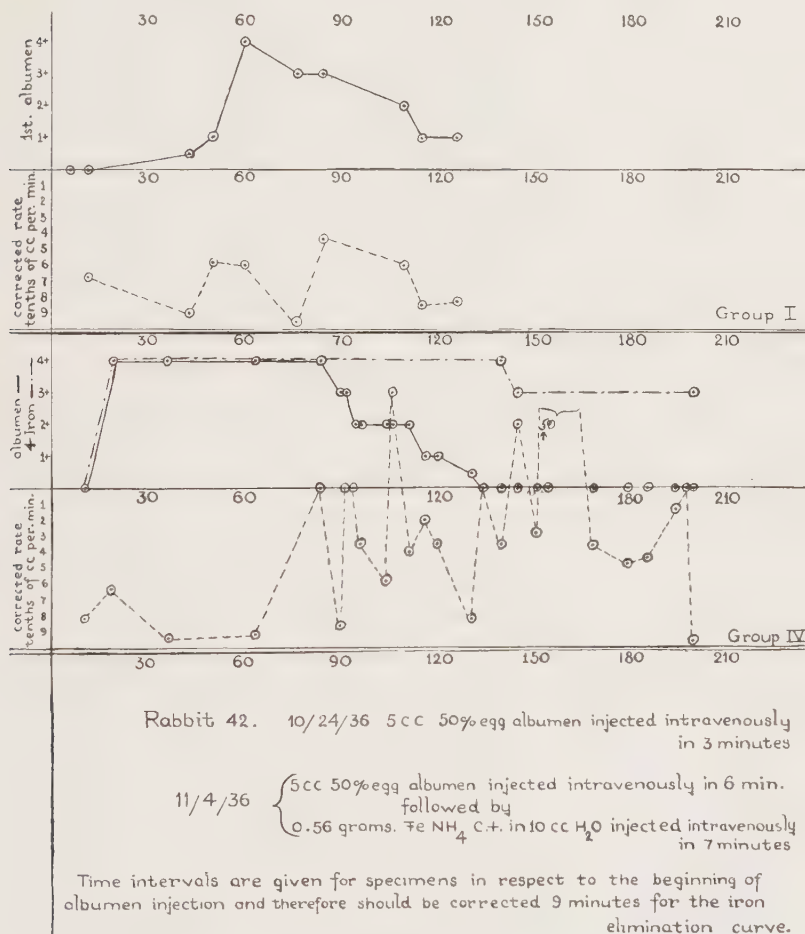


Figure 2

4. Out of nine animals injected with iron as a single injection only one animal failed to show iron in the urine when samples could be collected, in the first 20 minutes, but in only three animals was the maximum plateau attained in that time.

5. Out of seven animals injected with albumen and iron: 1) Albumen appeared in the urine twice within the first 20 minutes but no samples collected after 20 minutes were negative. 2) The maximum level of albumen elimination was somewhat sustained. 3) The albumen elimination curve was somewhat shortened. 4) Iron was present in all samples collected within 20 minutes and with one exception had reached the maximum plateau within that time. 5) The plateau of maximum elimination of iron was considerably lengthened.

Studies of the fixed tissue from the animals in group 5 which were sacrificed at 5, 8, 10, 13, 15, 20, 25 and 31 minutes after the beginning of the double injection, reveal that:

1. In the animals sacrificed at 5 to 20 minutes, the glomerular epithelium was continuous and swollen.

2. From 10 to 20 minutes the glomerular capillaries were dilated.

3. Coagulum appeared in the loops of Henle, distal tubules and collecting ducts within 5 minutes and from 10 minutes on was mixed with desquamated cells, cell debris and red blood cells.

4. Progressive degenerative changes in all the segments of the nephron occurred from 5 to 25 minutes. After this the various segments of some tubules appeared more normal.

5. In the 10-minute animal the capillaries in the medulla were ruptured and erythrocytes were extravasated into partially denuded collecting ducts and loops of Henle.

6. In the 13-minute animal there was considerable interstitial hemorrhage, the tissue spaces were enlarged and filled with coagulum; and coagulum, cell debris and red blood cells were found in the calyx.

7. Iron appeared in the proximal cells and in the glomerular capillaries in 3 minutes and increased in amount subsequently.

8. In 4 minutes iron was found in the lumen of all segments of the tubule.

9. In 10 minutes iron appeared in the swollen glomerular epithelium.



10. In 13 minutes iron and coagulum occurred in some glomerular spaces. In some the coagulum was mixed with fragmenting erythrocytes and cell debris.

11. From 15 to 20 minutes the iron and coagulum mixed with cell debris reached a maximum in the glomerular space.

12. After 20 minutes the capillaries were not as distended, the glomerular epithelium was not as swollen, and the content of iron and coagulum in the glomerular space was reduced.

13. At the height of degenerative changes in the various segments of the nephron (10 to 20 minutes) the coagulum and iron was less concentrated in the loops of Henle, the distals and in the collecting ducts.

#### SUMMARY

From the elimination curves it would appear that 1) egg albumen is not simply filtered through the glomerular membrane; 2) one injection of egg albumen delays the elimination of albumen following a second injection; 3) elimination of albumen reaches its maximum shortly after it is initiated; 4) when albumen and iron are injected together there is a greater elimination of both than when either is injected singly.

The histological studies of the elimination of albumen and iron when injected together indicate that albumen and iron are eliminated by the glomerular membrane coincident with progressive degenerative changes of it and of the tubular epithelium, and suggest that both albumen and iron are eliminated more freely through the damaged tubules, with a coincident loss of concentration of these substances by the tubules.

#### CONCLUSIONS

1. The elimination of egg albumen injected intravenously into rabbits is not a simple process of filtration through the glomerular membrane.

2. Iron, injected intravenously in the form of ferric ammonium citrate in combination with egg albumen, appears in the glomerular space following progressive degenerative changes in the glomerular membrane.

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## ABERRANT OVARIAN FOLLICLES IN THE IMMATURE RAT

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ONE PLATE (FOUR FIGURES)

After Hartman's review of the subject ('26) and many shorter papers (Engle, '27; Dederer, '34, among others) which have appeared on binuclearity of eggs and on polyovular follicles in mammals, another contribution to this subject might seem to be superfluous. The present author feels, however, that the material which he has collected from the study of an extensive series of rat ovaries provides some new observations. In the papers cited there has been no attempt made to find the proportion of the total ovarian follicles which contained abnormal ova. There are few observations on atypical eggs or follicles in the rat and these results should aid in extending our information concerning these structures to this species.

The collection of the material for this paper was incidental to a study of the influence of pituitary gonadotropic hormones on the follicular apparatus of the immature female rat (Lane, '35). It consists of 100 ovaries from sixty rats which varied in age from 15 to 64 days. In each of these ovaries the total number of follicles possessing more than a bilaminar granulosa was counted and follicular abnormality, when found, was recorded.

The rats in this series were distributed among the following age groups:

| <i>Number</i> | <i>Age,<br/>days</i> | <i>Number</i> | <i>Age,<br/>days</i> |
|---------------|----------------------|---------------|----------------------|
| 4             | 15                   | 3             | 38                   |
| 3             | 18                   | 3             | 39                   |
| 11            | 22                   | 4             | 44                   |
| 14            | 28                   | 4             | 45                   |
| 1             | 30                   | 2             | 56                   |
| 3             | 33                   | 3             | 64                   |
| 2             | 37                   | 2             | 66                   |

The ovaries were weighed, fixed, sectioned and counted as previously described (Lane, '35).

The total number of follicles counted in the 100 normal ovaries was 28,522. Of this total number there were five follicles (0.018%) containing binuclear ova; thirteen follicles (0.045%) were biovular, and three follicles (0.011%) which were triovular. These abnormal follicles represent only those which contained no obvious evidences of atresia. All the binuclear ova were contained in the ovaries of four rats, aged 22, 28 and 33 days. The polyovular follicles were more evenly distributed, generally occurring only once per ovary, although one of the ovaries of a 22-day-old rat, in addition to two binuclear ova, possessed also a triovular follicle. A study of a series of ovaries from animals treated with various hormone preparations (Amniotin, F.S.H., L.H.) indicates that the incidence of atypical follicles is not materially altered under these experimental conditions.

Since the atypical follicles were, with two exceptions, primary—i.e., non-antrum-containing—and since most of the aberrant follicles heretofore reported have also occurred in relatively immature follicles, it would seem not unlikely that the developmental abnormality which resulted in the formation of these structures might hasten their disappearance from the ovary. The usual course of development of these unusual follicles would seem to include atretic degeneration before the establishment of the antrum folliculi.

If this be true also for the adult female rat, it would then seem that much of the speculation concerning the fate of these ova in development is unwarranted since, in general, they probably do not develop to the point of ovulation.

#### CONCLUSION

In 100 ovaries from a series of sixty normal rats, in which every follicle over  $50\mu$  in diameter was studied and counted, there were 28,522 follicles. Of these, five or 0.018% contained binuclear ova; thirteen or 0.045% were biovular and three or 0.011% were triovular. Only two of these atypical follicles had progressed in development to the stage of formation of the antrum. This might indicate that atypical follicles are more susceptible to atretic degeneration before the appearance of the antrum folliculi than are normal follicles.

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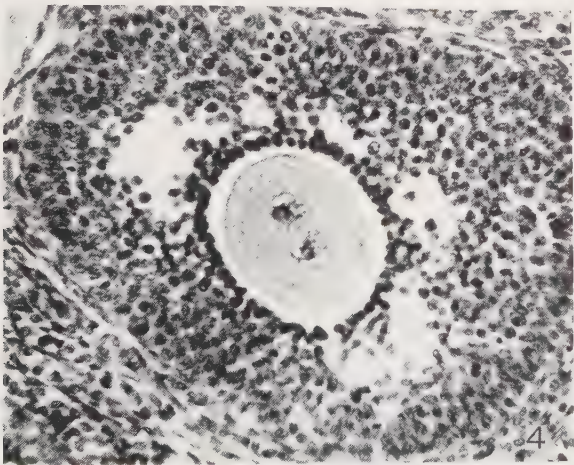
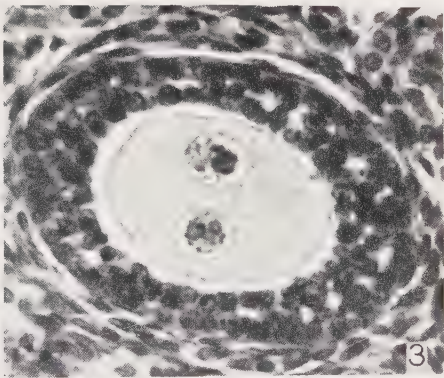
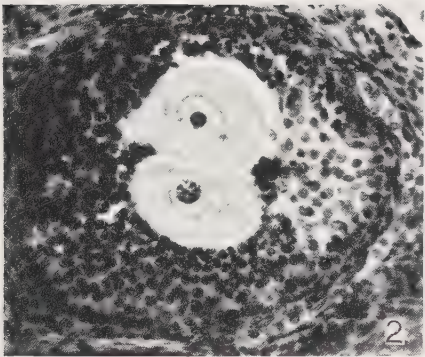
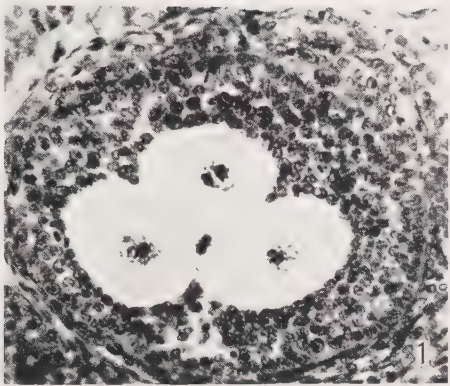
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## PLATE 1

### EXPLANATION OF FIGURES

- 1 Triovular follicle from the ovary of a 22-day-old rat.  $\times 350$ .
- 2 Biovular follicle from the ovary of a 37-day-old rat  $\times 350$ .
- 3 Binuclear ovum from the ovary of a 33-day-old rat.  $\times 450$ .
- 4 Binuclear ovum in a vesicular follicle found in the ovary of a 22-day-old rat.  $\times 352$ .





# HISTOLOGICAL CHANGES IN THE VESICAL MUSCLE FOLLOWING INJURY OF THE PERIPHERAL INNERVATION <sup>1</sup>

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ONE PLATE (NINE FIGURES)

## INTRODUCTION

Physiological experiments have dealt with vesical activity after section of the nerves innervating the organ. We have discussed bladder function after section of the preganglionic parasympathetic fibers, the posterior sacral roots, and the postganglionic sympathetic fibers. When the studies were completed, a certain number of the bladders were preserved for macroscopic and microscopic examination. The paper reports the anatomical findings in these specimens. We will attempt to show that there are characteristic changes in the morphology of the bladder after each of these operations. There is little available information dealing with the changes in smooth muscle after damage to its innervation. Mechanical factors, dependent upon difficulty in function of the organ, have a great part in the resultant anatomical abnormalities.

### *The structure of the bladder and urethra in a normal cat*

The bladder of the cat is unusual in that it rises freely into the abdominal cavity where it can easily be palpated. The urethra is from 4 to 10 cm. long depending upon the distension of the bladder. This mobility makes it possible to

<sup>1</sup> This study was supported by grants from the Rockefeller Foundation and from the John and Mary Markle Foundation.

store relatively large quantities of urine. Beside its attachment to the urethra the bladder is held in place by a ventromesial and two lateral peritoneal folds, by the ureters, nerves, and blood vessels. These peritoneal reflections appear short with the organ contracted, but are capable of great extension during filling. The ureters enter the dorso-lateral portion of the base of the bladder. The principle blood vessels and nerves run into the bladder at the base of the lateral mesenteric attachments. They permit considerable elongation as the bladder distends.

With the bladder filled the arrangement of the external muscle bundles can be seen. In the dorsal midline is a thick muscular band running longitudinally toward the vertex. Many other fibers pass transversely and interlace. On the lateral walls are two flat, wide muscular bands which send numerous offshoots toward the central band. Such an interlacing muscular arrangement is not present on the anterior wall. There are no prominent superficial longitudinal bands on the ventral surface. Fibers run obliquely from the region of the lateral mesenteric attachments toward the ventral midline near the vertex. At the vertex the longitudinal bands converge and form a prominent muscular mass.

When the bladder is opened the lining membrane appears as a flattened smooth surface if the viscus is filled, or is thrown into large folds if the organ is contracted. At the base two bands are seen converging from the ureteral orifices toward the urethra forming a Y-shaped structure. The base of the Y disappears in the urethra (fig. 7). The folds represent Bell's muscles or the continuation of the longitudinal muscles of the ureters. In man the muscle from the ureters on the two sides also fuses across the midline forming the trigone. This medial expansion is little developed in the cat so that the trigone is represented by the muscles of Bell.

Histologic study of the bladder wall disclosed four well defined layers; they are the mucosa, submucosa, muscular and serous. The mucous membrane consists of six to ten layers of transitional epithelial cells placed on a basement membrane.



The cells near the surface are flattened while the deeper layers are polygonal and contain large, deeply staining nuclei.

Beneath the mucous membrane is the submucous layer. Lying in the fibrous and elastic tissue may be seen numerous capillaries, small arterioles and venules, and occasionally a deeply placed and small pacinian corpuscle. Usually the submucosa appears as a very thin division between the epithelium and muscle, but with relaxation of the bladder it is thickened and forms the basis of the large papillae which project into lumen.

Two well-defined muscular layers are seen; the inner layer of fibers is circular while the outer layer runs longitudinally. The muscle bundles are rather closely knit but are divided at points by fibrous septa. Nerve trunks and blood vessels can be seen penetrating the muscle from the outside. The external longitudinal layer shows a greater tendency to be broken into bundles than the circular. The individual muscle fibers appear similar in all layers.

External to the muscular coat is a narrow band of connective tissue upon which rests the single-celled flattened layer of the serous peritoneal coat.

The ureters converge toward the base of the bladder as two small tubular structures. On cross section they show the same four layers. The mucosa is regularly thrown into four or five large folds which project into the lumen. The epithelium is still of the transitional type but is less differentiated than in the bladder. The submucosa, on the other hand is thicker. The circular muscle coat is well developed; the longitudinal layer is not as prominent in cross section. The individual muscle fibers are perhaps smaller than those of the bladder wall, and the sarcoplasm stains more deeply.

In longitudinal sections of the region of uretero-vesical junction the longitudinal muscular coat of the ureter is seen to continue beyond the orifice, sweeping downward in two muscle bands which lie internal to the muscle of the bladder wall and form Bell's muscles. Each is accompanied by a small artery. These two bands converge at the urethral orifice, and are continued downward in the cristal fold which

projects prominently into the lumen of the urethra. In the female cat this band of fibers can be followed to the external urethral orifice. In the male the fibers are more difficult to trace. They probably continue below the verumontanum at least as far as the external sphincter. Their relationship to the verumontanum requires further study.

The urethra also shows mucous membrane, submucosa, muscular and serous layers similar to those of the bladder and ureters. The dorsal surface of the urethra is thrown out into a tongue-like fold, the crista urethra, which we have described above. Indeed this fold almost fills the lumen of the relaxed urethra. The circular muscular coat is well developed throughout the length of the urethra. There are longitudinal bands about the whole circumference but most prominently along the anterior and posterior surfaces.

The vessels and nerves pass mainly anterior to the ureter and turn upward along the lateral border of the bladder after dividing into anterior and posterior branches. Schabadasch ('28) pointed out that the strands of sympathetic and parasympathetic fibers remain autonomous, although they form a plexus. The main mass of sympathetic fibers enter the bladder medial to the ureter whereas the parasympathetics lie lateral and make up the larger portion of the long nerves over the surface of the bladder. At the base are found the largest ganglionic masses; others are seen along the course of the nerves. On microscopic study even smaller groups of nerve cells are seen. While many of the ganglia lie just beneath the peritoneum, other large aggregations of cells are found buried deeply in the muscle bundles (fig. 9).

The pacinian corpuscles are frequently found in the subserous layer about the base of the bladder and around the terminations of the ureters. Others are present between the muscle fibers (fig. 8) and occasionally, unusually elongated forms occur in the depths of the submucosa.

*Histologic changes following bilateral section of the preganglionic parasympathetic nerves*

The sacral roots, which contain the preganglionic parasympathetic and certain of the sensory fibers to the bladder were cut in a number of cats. Sections from the bladders of five of these animals have been studied in detail; sections from the bladders of several others were examined. The physiological effects of the operation have been discussed in another place (Langworthy and Hesser, '36). Immediately following the operation there was retention with overflow of urine. The bladder held much larger quantities of fluid at low pressures. Later, after 10 to 14 days some function returned. The vesical volume was reduced to normal or to slightly less than normal. Rhythmical waves of contraction occurred in the vesical muscle, which showed no tendency to summation. They were effective in expelling small amounts of urine at one time; but there were no prolonged contractions sufficient to empty a large quantity of urine.

Palpation of the bladder through the abdomen gave the impression of an increased thickness of the wall. After the organ was removed, examination of its surface showed thickening of the external layer of the muscle; indeed the arrangement of the fibers could be seen clearly. When the bladders were sectioned, the hypertrophy of the entire wall was confirmed.

Infection easily develops after the parasympathetic fibers have been cut. This is particularly true if vesical readings are made at frequent intervals. Once the infection has developed it is difficult to control, due to the continuous presence of a residuum of urine.

The five specimens of the bladder were obtained from cats that survived from 6 to 49 days after cutting the sacral roots. All sections were stained with hematoxylin and eosin.

The changes in the two animals surviving 6 and 10 days were quite similar except for the evidence of cystitis found in the former. In this animal infiltration of leucocytes was present in the submucosa extending into the muscular wall. Here and

there denudation of epithelium was noted. The mucosa and submucosa in the 10-day survival showed no abnormalities. The bladder wall appeared somewhat thickened and the individual fibers increased in size. The nuclei of the fibers were normal but the demarcation of the sarcoplasm was indistinct. The cytoplasm was pale and rather granular. These changes were seen in both the circular and longitudinal bands of muscle but did not appear in the muscles of the ureters or Bell's muscles. The granular changes in the sarcoplasm were more marked in the 10-day survival. The 17-day specimen showed much the same changes, although here bands of muscle fibers could be seen projecting into the submucosa and some scarring of the epithelium was evident.

The most marked changes were observed in the two animals living 31 and 49 days after operation. In the first the epithelium appeared normal but in the second the mucosal cells had proliferated and a few cysts had formed. The usually flattened and flask-shaped cells of the normal vesical epithelium were now seen as polygonal in shape suggesting a rapid growth. The cell nuclei were large and stained deeply. Here and there masses had penetrated the submucosal layer and formed cysts (fig. 4). The muscular layers were greatly thickened. The individual smooth muscle fibers were large, the sarcoplasm again showed the pale granular changes observed in the previous preparations, and some of the inner bundles projected into the deeper portions of the submucosa. The blood vessels were more numerous and of larger caliber.

The impression gained was that the changes observed in the epithelium were related to irritation and infection. The striking findings were those seen in the muscle. The thickening of the muscle could be recognized as early as 6 days after the roots were cut. At first the fibers stained normally but the outlines of the cells were indistinct. Later the pale cytoplasm became granular. The hypertrophy was extreme 49 days after the operation. This was the longest survival period studied.



*Results of unilateral section of the preganglionic  
parasympathetics*

In several animals the sacral nerve roots were cut unilaterally. The operation produced a transient increase in vesical volume. After 2 weeks the volume returned to normal or less than normal (Langworthy and Hesser, '36). The cats failed to show any evidence of incontinence and apparently emptied the bladder quite normally. No examination was made for a possible residuum of urine. On the other hand, the bladder wall became thickened. This thickening could be observed even on palpation through the abdominal wall.

At the close of the experiments the abdomen was opened and the bladder exposed. When the bladder was only moderately distended it was at once clear that the development of the muscle was greater on the side where the nerve had been sectioned. With moderate distension the folds in the vesical muscle were more marked on the abnormal side.

This difference was observed with the eye in sections of the fixed bladder; it was even more evident in the histological preparations. An example is shown in figure 1 of an animal that survived 3 weeks. The arrow points to the mid-line of the bladder. The sacral nerves supplying the portion on the right had been cut 20 days previously. The total size of the bladder was increased on the right. The lumen was larger, and both the longitudinal and circular layers were thicker. The submucosa on the right was thrown into higher folds than on the left. The changes in the muscle on the operated side were similar to those described in the previous section.

*Changes in the bladder following section of the posterior  
sacral nerve roots*

Studies were made of the bladders of five animals following section of the posterior sacral nerve roots. The survival periods extended from 9 to 170 days. In the case of two of the cats the sympathetic innervation of the bladder was severed subsequent to the section of the posterior roots.



After section of the posterior sacral roots, the parasympathetic reflex arc failed to function due to the loss of the sensory fibers (Dees and Langworthy, '35). The bladder became overdistended, holding fluid at low pressures without the occurrence of rhythmical waves. The cats later developed overflow incontinence which continued as long as they lived. Cystitis was almost inevitable following catheterization, as the bladder never emptied. Attempts were made to empty the organ passively by pressure upon the abdomen. Under these circumstances it was difficult to force out all the fluid, for the wall remained inert. On palpation through the abdomen the bladder wall felt thin and yielding.

Sympathectomy performed on cats after section of the posterior sacral roots resulted in the appearance of a few rhythmical waves during filling and also in partial emptying of the organ. The bladder became smaller.

The first animal survived only 9 days after the operation. There was a membranous cystitis. On histological examination small cyst-like outgrowths of the epithelium were seen throughout the mucous membrane of the bladder and urethra. They appeared also in the lower ends of the ureters. The submucosa and even the spaces between the muscle bands were infiltrated with leucocytes.

The muscle fibers showed obvious degenerative changes. The sarcoplasm of the individual fibers appeared broken up into granular ground-glass like masses which were more or less fused. The nuclei of the cells were swollen and stained poorly. There was no evidence of division of the muscle fibers. The ends of the individual fibers were atrophic. The musculature of the urethra showed similar but less marked changes; the crista muscle was normal.

The bladder of an animal surviving 33 days showed fewer changes than the previous preparation. This was no doubt due to lack of infection. The mucosa was undamaged and cystitis was not present. The general appearance of the muscle fibers was similar to that seen in the previous preparation. Again the fibers appeared friable with the breaking down of the

sarcoplasm into glairy globules. The nuclei were enlarged and very pale. The muscle of the urethra was somewhat atrophic. The crista muscle was normal; the crista itself was flattened. Another cat surviving 54 days shows essentially the changes described in the previous two. Here again there was cystitis with evidence of epithelial proliferation, and the picture of muscular atrophy.

The third animal lived 44 days after section of the posterior sacral roots. The abdominal sympathetic chain was removed bilaterally 18 days before death. The bladder was small and contracted. The histological changes in this animal proved the most interesting of the whole series. There was no infection and the epithelium and submucosa were normal. The bulk of the muscle was increased and attempts at muscle hypertrophy were clear (fig. 3). The new growth was marked both in the bladder and urethra. The appearance was unusual due to the fact that the nuclei were arranged in regular order across the whole of a muscle band (fig. 3). The cells appeared not to have matured and were not separated from each other (fig. 3). The nuclei looked normal, and there was sarcoplasm around them. However the ends of the muscle fibers were frayed and thin. The stained areas of the sarcoplasm showed the ground-glass granulation noted in the previous preparations. The urethral muscle was also hypertrophied. The crista was large and completely filled the lumen of the urethra.

The longest survival was 170 days; the cervical sympathetic trunks were removed 161 days before death. This long period of life following section of the posterior roots was dependent upon the sympathectomy which permitted some degree of vesical function. The bladder showed considerable evidence of cystitis. There was hyperplasia of the epithelial cells with disappearance of the flattened superficial cell layers and the formation of cysts. The submucosa was infiltrated with leucocytes. The muscle in this case showed an extreme degree of atrophy. The fibers in a single bundle were separated by considerable spaces. The individual muscle fibers were small with pale nuclei and granulated cytoplasm. The

ends of the fibers were thin and narrow. The ureters appeared normal. There were no sections of the urethra.

The muscles of all the bladders after section of the posterior sacral roots showed signs of atrophy. In one instance where a sympathectomy was performed secondarily only 18 days before death there was evidence of some attempt at cell division.

*Changes in the bladder after section of the postganglionic sympathetic fibers*

The abdominal sympathetic chains were exposed extra-peritoneally by a lateral incision. Removal of the post-ganglionic fibers upon one side caused a sharp decrease in vesical volume (Langworthy, Reeves and Tauber, '34). Later, over a period of weeks, the volume tended slowly to increase again and to approximate although it did not reach the normal. Removal of the sympathetics bilaterally produced a more marked and lasting decrease in vesical volume. The bladder remained in the pelvis and no longer rose into the abdomen.

The cats were killed and the bladder was studied histologically 6, 12, 26, 28 and 64 days after unilateral section of the post-ganglionic sympathetic fibers. The unilateral operation gave a control preparation of the unoperated side. The histological picture was similar in all and well established on the sixth day. The lumen of the ureter was dilated on the side of the nerve section so that the mucosal folds were obliterated (figs. 5 and 6). The submucosa and mucosal layers appeared flattened around the lumen. Similarly the orifice of the ureter was dilated upon the operated side. On the unoperated side the ureter was quite normal.

There was a marked vasodilatation of the blood vessels in the ureter, bladder and urethra upon the affected side. This was apparent on the sixth day and quite as evident at the end of 2 months. The dilatation of the blood vessels was for the most part unilateral. However at the neck of the bladder where the Bell's muscles converge, vascular dilatation could often be observed on both sides, although usually more marked

on the operated. There is some evidence that there is a crossing of nerve fibers from one-half of the bladder to the other in this region. The vasodilatation was seen best in the depths of the submucosa where the arterioles and capillaries were engorged with blood.

There is reason to believe that the sympathetic nerves contribute largely if not exclusively to the innervation of the ureters, Bell's muscles and crista of the urethra. However, no clear-cut changes could be observed in this muscle. In certain of the preparations the muscle on the abnormal side appeared to be undergoing hyaline changes. There was no real evidence of attempts at hypertrophy on the part of the muscle. However the crista projected prominently into the lumen of the urethra. The bladder remained in the pelvis and it was our impression that the urethra was shortened.

#### DISCUSSION

These experiments show the response of vesicle muscle to injury of the nerve supply. After section of the preganglionic parasympathetic fibers the muscle shows a tendency to hypertrophy. There is increase in the size of the individual fibers as well as in the total bulk of the muscle. It appears that the muscle attempts in this way to compensate for the trauma and carry on the increased work. Unilateral section of the preganglionic parasympathetic fibers leads to hypertrophy which is more marked on the side of the nerve lesion. The injured side has the greater burden of increased work and shows the greater response in compensation.

When the posterior sacral roots are cut without injury of the parasympathetic nerves, the bladder no longer empties, and becomes dilated by the accumulation of urine. The stretching of the wall and interference with the blood supply leads to an atrophy of the smooth muscle fibers. Subsequent section of the postganglionic sympathetic fibers causes the dilated bladder to become smaller and a certain degree of contractility returns in the muscle. Under these conditions attempts at muscular division occur.



We have cut the postganglionic sympathetic innervation of the bladder and allowed the cats to survive for periods of 1 week to 2 months. The abdominal sympathetic chain has been removed either on one or both sides. The lumen of the ureter is dilated and no longer shows the usual folds, and the ureteral orifice becomes enlarged on the side where the sympathetic fibers have been sectioned. Moreover a vasodilatation can be observed in the ureters, bladder and urethra. If the sympathetics are cut on one side the vasodilatation is unilateral.

There is no evidence of change in the vesical muscle after injury of the sympathetics. The urethra tends to be shortened and Bell's muscles project prominently into the lumen but they do not seem hypertrophied. It is probable that the ureters, Bell's muscles and the crista of the urethra are innervated largely if not exclusively from the sympathetic nervous system. It must be kept in mind that we cut the preganglionic parasympathetic and postganglionic sympathetic fibers.

The findings in terms of hypertrophy and atrophy appear to be dependent on mechanical factors. The injured muscle attempts to compensate by hypertrophy in order to carry on contraction efficiently. Atrophy intervenes when the muscle fibers are put under abnormal pressure and stretch over a period of time.

The changes in the epithelium of the bladder are related in large measure to infection. The epithelial cells begin to proliferate actively, forming pearls and finally true cysts. It is perhaps of interest to show that cystitis cystica can be produced experimentally in this way.

The only nerve endings which can be recognized in the bladder with ordinary hematoxylin and eosin stains are the pacinian corpuscles. They are most abundant at the base of the bladder and at the upper end of the urethra. Often they are found on the lateral surfaces near the entrance of the ureters into the bladder. They are common just beneath the peritoneum, but they also occur buried in the vesicle muscle. Their shape varies enormously.



Elliott ('07) stated that he removed the postganglionic parasympathetic supply to the bladder by picking away the ganglia and pacinian bodies from the wall with a fine forceps as well as tearing off the vesical plexus. Contractions of the bladder still occurred. Later a 'torpid overgrowth' took place but previous to this the muscle was supple and quick to respond to electrical stimulation. Finally it had no rhythm and only dull irritability.

We have shown in the figures a ganglion buried deeply between the muscle bundles. Small groups of cells or even individual nerve cells were frequently found in the muscle or lying almost at the edge of the submucosa. It is impossible that total denervation can be carried out by the method followed by Elliott. Only the larger ganglionic masses and pacinian corpuscles near the base of the bladder are accessible to easy discovery and removal. Any attempts at more radical search with forceps and hand lens must end in direct damage to the blood vessels and to the muscle itself. No doubt the denervation as performed by Elliott was reasonably complete.

Elliott ('07) observed the hypertrophy of the vesical muscle after unilateral section of the preganglionic parasympathetic fibers. He did not notice that the increase in the muscle was greater upon one side. He found that the muscle on the side of the nerve section was unusually irritable to stimuli such as electricity and cold.

#### SUMMARY

Following section of the preganglionic parasympathetic fibers the smooth muscle of the bladder and urethra hypertrophies. If the nerves are cut on one side the hypertrophy is greater on that side. We feel that this hypertrophy is an effort at compensation. Section of the posterior sacral roots which carry proprioceptive impulses from the vesical muscle abolishes contraction waves, and leads to persistent stretching of the muscle. Atrophy of the smooth muscle fibers follows.

However if the sympathetic fibers are cut later in these cases so that the bladder becomes smaller, attempts at multiplication of fibers appear. Section of the postganglionic sympathetic fibers leads to dilatation of the ureters and vasodilatation of the blood vessels of the ureters, bladder and urethra.

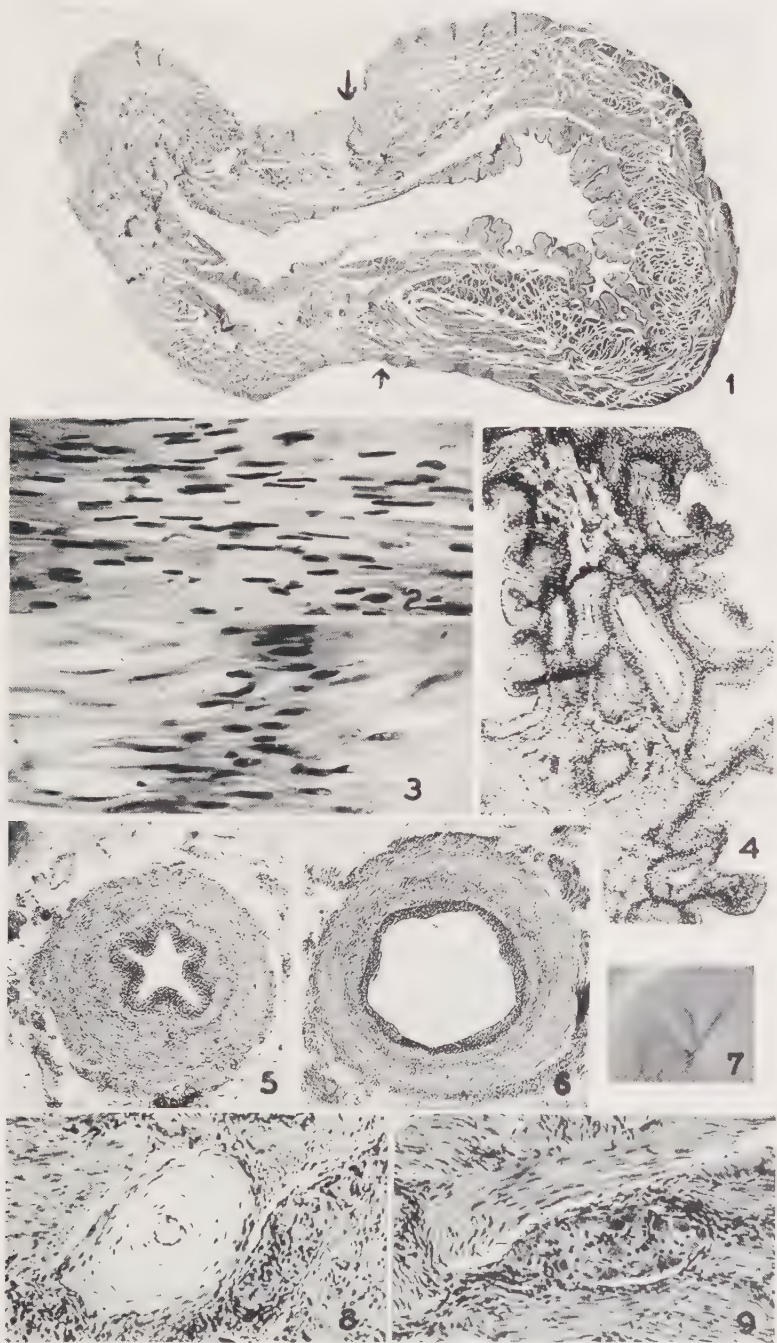
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#### PLATE 1

##### EXPLANATION OF FIGURES

- 1 A cross section of the bladder wall. The sacral nerve roots were cut on the right side 3 weeks before death. There is hypertrophy of the right half of the bladder.  $\times 6$ .
- 2 Normal vesical muscle.  $\times 300$ .
- 3 Vesical muscle from a cat living 49 days after section of the posterior sacral roots and 18 days after removal of the abdominal sympathetic nerves.  $\times 300$ .
- 4 Cystitis with proliferation of the epithelium and the formation of cysts in an animal whose sacral roots had been cut for 49 days.  $\times 50$ .
- 5 Section of a normal ureter of a cat.  $\times 32$ .
- 6 The abdominal sympathetic trunk was removed on the side of the body opposite to the normal ureter shown in figure 5, 28 days before death. The ureter on the side of the sympathectomy is dilated as illustrated here.
- 7 The base of the cat's bladder showing Bell's muscles arising at the ureteral orifices, converging, and extending downward into the urethra.  $\times 4$ .
- 8 A pacinian corpuscle embedded in the vesical muscle of a cat.  $\times 106$ .
- 9 A group of autonomic cells in the vesical muscle of a cat.  $\times 106$ .





# THE DEVELOPMENT OF THE SUPERIOR CAVAL SYSTEM IN THE RAT

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FOUR FIGURES

The great anterior blood vessels of mammals were among the first to be studied both by reason of their great size and of their location next to the heart. Among these, the superior vena cava has received a considerable share of this interest. While the development of the caval system in man has been investigated rather thoroughly, corresponding investigations on the lower mammals are, for the most part, incomplete. This study has been attempted in the hope that a description of the development of the superior vena cava in the rat may be helpful, both in further understanding its development in man, and in furnishing a basis for a comparative study of the development of the great anterior veins in the Mammalia.

It gives me great pleasure to be able to express publicly my deep appreciation for the help of Dr. E. M. Shearer without whose aid this work would never have been attempted. Many thanks are also due to Doctor Shearer for his kindness in lending the author his collection of rat embryos upon which this study is based.

The classical work on the development of the superior vena cava was published in 1850 by Marshall. In tracing the growth of the embryonic human venous system he reported that the remnants of the left anterior cardinal vein in the adult formed, outside the pericardium, the left superior intercostal vein and that, inside the pericardium, it formed the oblique



auricular vein (since called the oblique vein of Marshall). The left anterior cardinal vein also helped form the coronary sinus. Cameron ('15) reported that there is normally present in the adult human, a small venule which runs from the left superior intercostal vein to the pericardium and then runs in the vestigial fold of Marshall for a short distance. This, he says, is a remnant of the extrapericardial part of the left anterior cardinal.

This study was carried out on fifteen embryos of the albino rat, *Mus norvegicus albinus*. These were embryos of accurately timed age, which had originally been obtained from The Wistar Institute and mounted in series of transverse

TABLE 1  
*Embryos used in the study*

| <i>Litter no.</i> | <i>Age of embryos</i> | <i>Section thickness<br/>(in <math>\mu</math>)</i> | <i>Number<br/>sectioned</i> |
|-------------------|-----------------------|----------------------------------------------------|-----------------------------|
| XII               | 12 days, 11 hours     | 15                                                 | 2                           |
| X                 | 12 days, 23 hours     | 15                                                 | 2                           |
| VI                | 13 days, 3 hours      | 15                                                 | 1                           |
| XI                | 13 days, 11 hours     | 15                                                 | 1                           |
| V                 | 14 days, 1½ hours     | 15                                                 | 1                           |
| XIII              | 14 days, 3 hours      | 15                                                 | 1                           |
| VIII              | 14 days, 11 hours     | 20                                                 | 1                           |
| III               | 15 days,              | 15 and 20                                          | 2                           |
| IV                | 15 days, 20 hours     | 20                                                 | 2                           |
| VII               | 16 days,              | 20                                                 | 2                           |

sections, after fixation in Bouin's fluid. All had been stained with Mallory's triple connective tissue stain. A summary of the embryos used in this study will be found in table 1. Litters had been numbered in the order in which they were obtained but, for convenience, have been listed in the table in the order of age. During the course of the study three embryos were reconstructed in wax after the method of Born. The anatomy of the adult rat was studied on specimens in which the arteries had been injected with a gelatin mass, so that the veins were, for the most part, gorged with blood.

The arrangement of the adult veins has recently been described by Greene ('35). My own observations are completely

in accord with hers. The external jugular vein of the rat is relatively much larger than in man, draining a large portion of the head and neck. It joins the subclavian where that vein crosses the first rib. The junction of the relatively small internal jugular and the subclavian forms the superior vena cava (on either side). The two *venae cavae superiores* enter the right atrium separately. The internal mammary and vertebral veins of either side drain into the vena cava superior of the corresponding side. The left superior cava, near its point of entrance to the right atrium, receives the azygos vein. The azygos receives all the intercostal veins of both sides with the exception of the upper three or four right intercostal veins, which drain into the right superior cava, either directly or by means of a right superior intercostal vein; and the lower two or three right intercostals which drain into the azygos via the hemiazygos vein.

The earliest stage reconstructed was a litter of embryos of 12 days and 23 hours (fig. 1). At this stage is represented the cardinal venous plan which is characteristic of all mammalian embryos. Blood from the head is returned to the heart by means of the large anterior cardinal veins. Running anteriorly through the body, carrying blood from the more caudal regions, are the paired posterior cardinal veins, each of which joins the corresponding anterior cardinal to form the common cardinal veins or ducts of Cuvier, which empty into the sinus venosus of the heart. The posterior cardinals receive a series of segmental veins which accompany the segmental branches of the dorsal aorta and the spinal nerves. Some of the cervical segmentals join the anterior cardinal and the rest join the posterior cardinal. It will be seen in later stages that there is a gradual anterior shifting of the segmental veins, so that eventually not only all the cervical but even the upper thoracic veins come to drain into the anterior cardinals. At this stage the drainage from the anterior limb bud (future subclavian vein) is into the posterior cardinal just caudal to the entrance of the latter into the common cardinal vein. This also shifts anteriorly in development. This shifting process is

## ABBREVIATIONS

ao.dors., dorsal aorta  
 v.card.ant., anterior cardinal vein  
 v.card.post.sin. or dext., left or right  
 posterior cardinal vein  
 v.cav.inf., inferior vena cava

v.cav.sup.sin. or dext., left or right supe-  
 rior vena cava  
 v.jug.ext., external jugular vein  
 v.seg.cerv. IV, fourth cervical segmental  
 vein  
 v.subel.sin., left subelavian vein

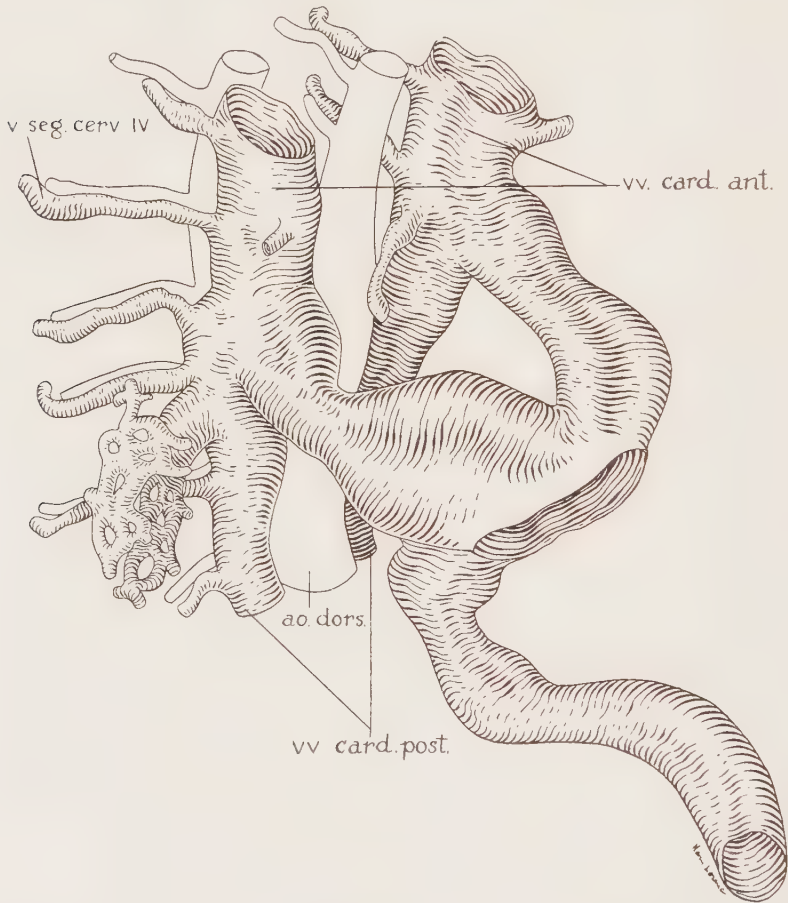


Fig. 1 Reconstruction of the anterior veins of an embryo of 12 days, 23 hours, ventrolateral view.

due to the caudal migration of the heart, which, of course, draws with it the ducts of Cuvier.

An earlier stage (an embryo of 12 days and 11 hours) was studied. In this series the anterior cardinals are shorter for the heart is located more cephalad. It is interesting to note that, at this stage, all except the first two cervical segmental veins enter the posterior cardinal, only these two joining the anterior cardinal. The entrance of the subclavian anlage is also more caudal than in the later stage, entering the posterior cardinal well away from the beginning of the common cardinal.

In embryos of 13 days and 11 hours the heart has migrated caudally and there has been a corresponding increase in the length of the anterior cardinal veins. At this stage, the right posterior cardinal vein is, for the first time, appreciably smaller than the left. The segmentals received by the anterior cardinals are still from the cervical regions and the thoracic segmentals drain into the postcardinals. These latter are the veins which will eventually become the intercostal veins and which, in the adult, join the azygos vein.

In an embryo of 14 days, 1½ hours (fig. 2), the ribs are, for the first time, clearly visible. The rat, in contrast to most mammals, has thirteen ribs and, therefore, twelve intercostal spaces, each drained by an intercostal vein and supplied by an intercostal artery which takes origin from the aorta. The highest segmental vein in the thoracic series (which will later become the first intercostal) joins the posterior cardinal immediately behind the entrance of the postcardinal into the duct of Cuvier. At this stage the subclavian veins have come to drain into the anterior cardinals. In one embryo the first intercostal vein of the left side has already come to drain into the anterior cardinal vein. This is the only intercostal vein to drain into the anterior cardinal vein in this series. Both posterior cardinals have now greatly diminished in size and importance, due to the development of the inferior caval system. This has been described by Butler ('27). The diminution of the posterior cardinals is not, however, equal on the

two sides. On the left side, though relatively smaller than in earlier stages, it is still easily traceable throughout the length of the thoracic region, lying to the left of, and slightly posterior to, the aorta and receiving the left thoracic segmentals. These latter may now be regarded as the intercostal veins, and the left posterior cardinal as the definite anlage of the azygos vein. The posterior cardinal on the right side has become extremely small and difficult to trace except at its anterior end where it joins the duct of Cuvier. It is, in fact,

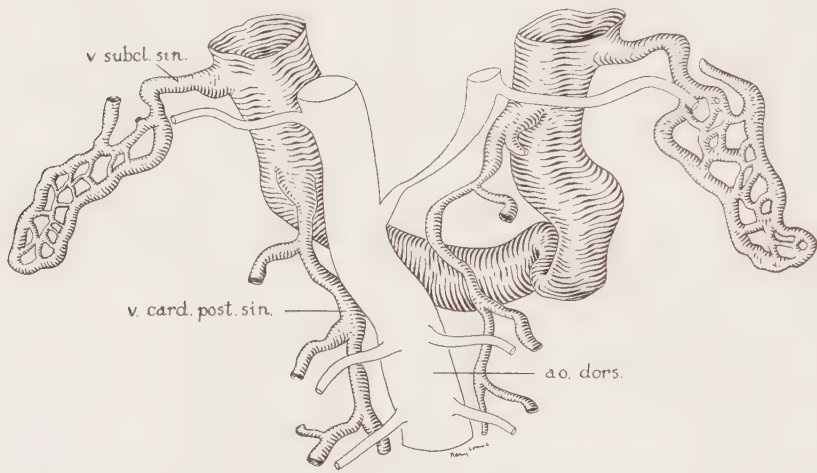


Fig. 2 Reconstruction of the anterior veins of an embryo of 14 days, 1½ hours, dorsal view.

impossible to trace it at all in most cases, and it is difficult to understand what becomes of the blood in the right intercostals, since at this stage there seems to be no connection between them and the left posterior cardinal or azygos vein. Probably this matter can only be completely cleared up by study of injected specimens which unfortunately were not available. At this stage, the external jugular has appeared as a small vessel joining the anterior cardinal at a point some distance anterior to the junction of the subclavian with the anterior cardinal.



An embryo of 14 days and 11 hours (fig. 3) is an important stage in the development of the azygos system, since it shows clearly the appearance of a venous plexus dorsal to the aorta in the lower thoracic region, through which the right intercostal veins come eventually to drain into the azygos. This plexus apparently represents all that there is in the rat of

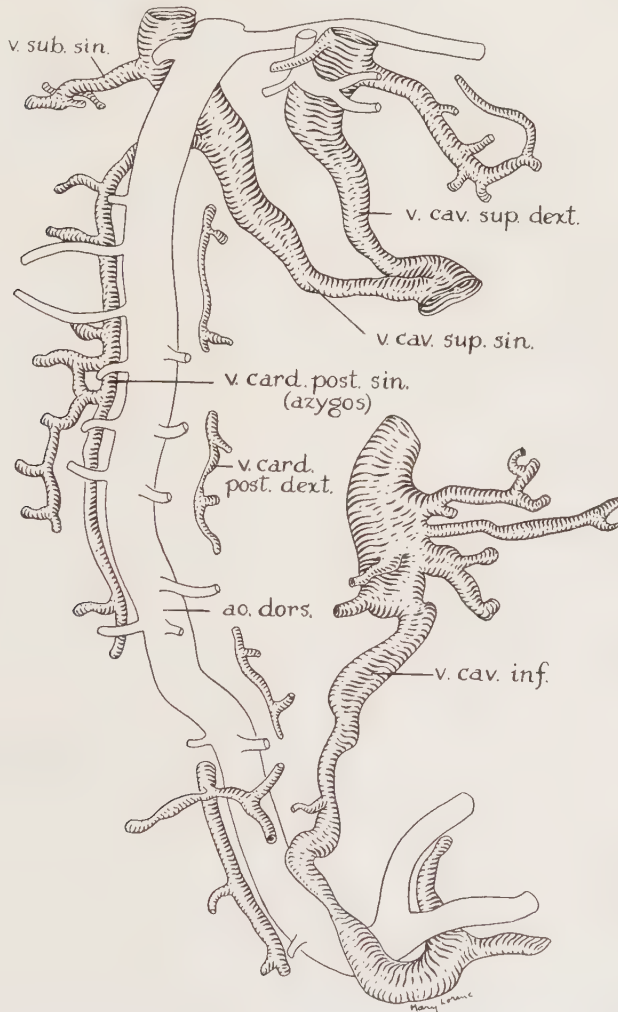


Fig. 3 Reconstruction of the veins of an embryo of 14 days, 11 hours, dorso-lateral view.

the supracardinal veins which are characteristic of most mammals. The lowest intercostals of both sides drain into paired channels derived from this plexus, both of which channels end in the left posterior cardinal vein, which we may now call the azygos. The terminology of the more anterior portions of the caval system may now also be adapted to the adult form. It is apparent that the anterior cardinal on either side, anterior (cephalad) to the point at which it is joined by the subclavian, represents the internal jugular of the adult. Caudal to this junction it may now be called the superior cava. On the left side the superior vena cava is represented by the anterior cardinal vein from its junction with the subclavian to the point at which the azygos (former posterior cardinal) joins it, and from here to its entrance into the heart by the former duct of Cuvier. On the right side also the superior vena cava is derived from the anterior cardinal and duct of Cuvier, but the distinction between these two original segments is not so clear, due to the disappearance of the right posterior cardinal.

In an embryo of 16 days, the veins have reached practically the adult condition. The azygos vein receives the hemiazygos vein in its lower portion, while the anterior portion receives the fourth to the tenth intercostals of both sides. This anterior portion of the azygos is derived directly from the left posterior cardinal vein. The caudal portion, together with the hemiazygos seems to be a derivative of the dorsal aortic or supracardinal plexus. The two embryos of this litter show that the azygos and hemiazygos are of approximately the same size. The hemiazygos ascends to the right and the azygos to the left of the vertebral column and each receives the last few intercostals of the ipsilateral side. In embryo VIIa the azygos received the hemiazygos in the eleventh space and each received the eleventh and twelfth intercostals from its own side. In embryo VIIb the hemiazygos entered in the tenth space and each drained the last three intercostal spaces of its own side. The dissection of several adults also revealed the junction of the azygos and hemiazygos to be in the tenth

or eleventh intercostal space. This embryo (VIIb) is of further interest in that it shows the manner in which the external jugular vein is shifted from its original point of drainage into the anterior cardinal (internal jugular) to its adult condition of joining the subclavian (fig. 4, D). On the right side of this embryo, the external jugular joins the subclavian,

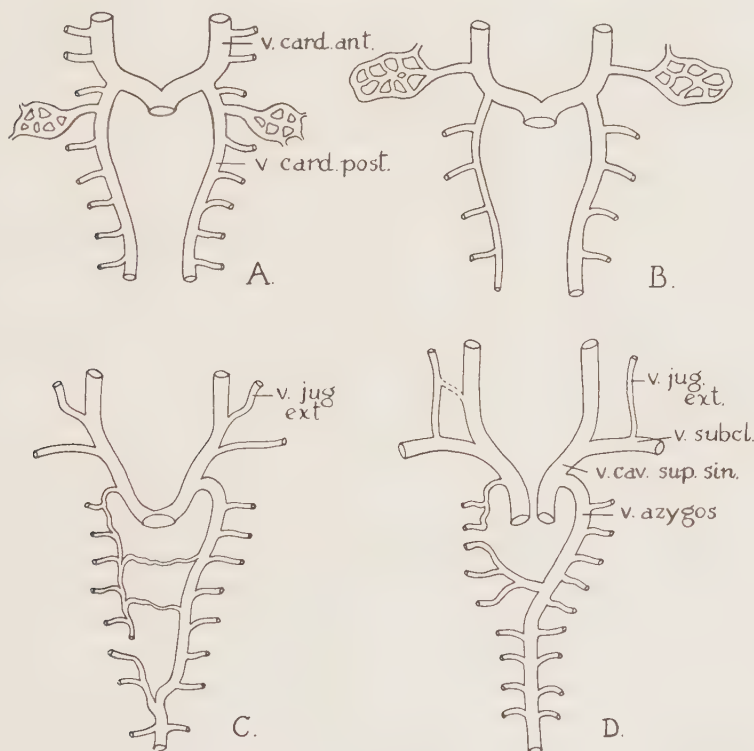


Fig. 4 Diagrammatic schema of development, ventral view. A, about 13 days; B, about 14 days; C, about 14½ days; D, about 16 days.

but, on the left side, the manner of this change is explained by the presence of a venous loop by which the external jugular communicates with both internal jugular and subclavian veins. Apparently the terminal part of the external jugular of the adult is a new formation, which causes its original communication with the internal jugular to drop out.

The development of the great anterior veins in the rat follows a much simpler course than the corresponding development in man. Butler ('27) has shown that this is also true in the development of the inferior vena cava due to the absence of the supracardinal veins. He reports that, while there are no definite supracardinal veins in the rat, there is, at one time, a definite supracardinal venous plexus. This absence of the supracardinal as a pervious venous channel results in a different method of formation for the azygos vein than is shown in man. In humans, the azygos develops mostly from the supracardinal vein and only its terminal part develops from the postcardinal. In the rat only a very small portion of the azygos vein comes from the supracardinal plexus and most of it is a direct derivative of the left posterior cardinal vein. The development of the superior vena cava is much simpler than in man because the adult rat presents a much less advanced type of venous system with a double superior vena cava the normal condition. The development of the superior vena cava in man may be described as follows:

The veins of the head and neck are derived from the anterior cardinals, which are, at first, paired and symmetrical but are comparatively short and receive blood from the cervical region through segmental branches which belong only to the most cranial of the cervical segments. The other segmental cervicals (including the subclavian veins) open at first into the posterior cardinals. Later the anterior cardinal veins become elongated and the segmental cervicals, including the subclavian, open into them. Next an anastomosing vessel is formed obliquely across from a point on the left cardinal opposite the entrance of the subclavian vein to a point nearer the heart on the right cardinal. That part of the left cardinal cranial to the entrance of the subclavian becomes the left internal jugular. The anastomosis forms the left innominate vein. That part of the left cardinal between the entrance of the subclavian vein and the duct of Cuvier, the duct itself and part of the left horn of the sinus venosus form the coronary sinus. On the right side the more distal portion of the

anterior cardinal forms the internal jugular, that part which runs between the entrance of the subclavian and the anastomosing vein becomes the right innominate and the common stem formed by the latter and the left innominate becomes the superior vena cava which opens into the right atrium. The external jugular appears on each side later than the anterior cardinal as an independent vessel which lies parallel to the internal jugular vein and opens into it near the entrance of the subclavian. This opening later shifts to the subclavian vein itself, where it is found in the adult. The vestigial fold and the oblique vein of Marshall are all that usually remain in the adult of the left superior vena cava. The right superior cava, within the pericardium, passes in front of the right pulmonary vessels and the left has a similar relation (it is bound to the pulmonary vessels by a fold of serous pericardium). When the left superior vena cava atrophies, the pericardial reflection remains in front of the left pulmonary vessels. This is the vestigial fold of Marshall. The intrapericardial portion of the left superior vena cava becomes the oblique vein which turns around the left auricle to end in the left horn of the sinus venosus (coronary sinus). The extrapericardial part of the left duct of Cuvier joins the superior intercostal vein.

It will be noticed that development in the rat follows very nearly the same pattern. The adult rat presents an anterior venous system very much like that of a human embryo of about 4 months. The superior vena cava of each side is developed from the terminal segment of the anterior cardinal vein and from the duct of Cuvier. The azygos vein arises principally from the left posterior cardinal, but its caudal portion, together with the hemiazygos, is derived from a new plexus comparable to the supracardinal veins of other mammals. The segmental veins of the thoracic series (which are destined to become the intercostal veins of the adult) empty at first into the postcardinal and then shift their position cephalad so that the three most anterior veins of the series open into the anterior cardinal vein. The subclavian vein



also shows this cephalic migration for its origin is also from the postcardinal, changing later to the anterior cardinal vein. In the final metamorphosis to the adult condition the first three (usually) intercostal veins unite to form the superior intercostal vein which, on the left side, empties into the azygos vein and, on the right, into the superior vena cava. The superior intercostal vein of either or both sides may be lacking so that, even on the left side, the two or three highest intercostals may drain directly into the vena cava superior.

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## THE VASCULARITY OF THE HYPOPHYSIS OF THE FROG (*RANA PIPIENS*)

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### FOUR FIGURES

This study was undertaken by the first-named author as part of a comparative investigation of the vascularity of the hypophysis in representative vertebrates. As circumstances obliged him to abandon the work when he had prepared and examined nine of the specimens used and written a preliminary note regarding them, the second author had some further material prepared, repeated most of the measurements, and wrote the present report.

The anatomy and physiology of capillaries in glands of internal secretion and quantitative data regarding them would appear to be of particular interest, and since such studies upon the vessels of the central nervous system have been carried on in this laboratory for some time, it seemed worth while to extend them to the hypophysis, an organ which has recently received so much attention.

The hypophysis of the elasmobranch was found by Sterzi ('09) to have in the anterior lobe large meshes of wide sinusoids, while the dorsal lobe has finer meshes formed by slenderer vessels. The area hypophysaria, which contains the forerunner of the pars nervosa of higher animals has a very rich superficial net but no vessels in its substance.

According to Schöbl (1878) the peculiar looped arrangement of the vessels which he observed to exist instead of a capillary network in the brains of the majority of lizards is present in the hypophysis of these animals also. This is not true in

amphibians, however, where the Caudata have capillaries forming non-anastomosing loops in the central nervous system but a spongy network in other tissues, including those of the hypophysis (Craigie, '38 a). The neural lobe in the latter animals has only a superficial capillary network, which dips into the folds of the lobe but does not penetrate its substance. In Salientia the capillaries have a three-dimensional reticular arrangement both in the central nervous organs and in the hypophysis.

Tilney ('11) stated that in the juxta-neural portion (pars intermedia and pars tuberalis) of *Rana sylvatica* "there is a conspicuous absence of all blood-vascular elements" while in the distal portion the vascularization is rich.

Atwell ('18), however, states that in the frog (*R. catesbiana*, *R. pipiens*) the pars intermedia is fairly vascular while the pars tuberalis is non-vascular.

Herring ('08) noted that in a variety of mammals the anterior lobe of the hypophysis has extremely numerous and wide vessels like liver sinusoids, the posterior lobe has true capillaries resembling in number and arrangement those in the white matter of the brain, while the intermediate lobe is very poor, having a few capillaries in the cat, slightly more in the dog, but none in the monkey. Stendell ('14) confirmed Herring's results. Herring ('08 a) stated further that the mode of ontogenetic development of the vessels in the anterior and posterior lobes differed, those in the anterior one being sinusoidal in origin while those in the posterior one grow in from the periphery as do the vessels of the central nervous system.

Tilney ('13) indicated that in various mammals and the domestic fowl the pars tuberalis is a little more vascular than the pars intermedia and considerably less so than the pars anterior.

Brown ('24) found that in the rat the pars nervosa has a close network of sinusoids, while the pars anterior has a wider-meshed network of vessels twice as large, that the pars intermedia is poor in vessels except at the periphery, and that the pars tuberalis is well supplied.

De Beer ('26) states that the pars anterior is highly vascular in all vertebrates and gives drawings of sections of the organ in haddock, frog, fowl, and cat which make the vascularity appear to increase in that sequence. In the frog the vessels in all lobes are much wider than in the other three species mentioned and the neural lobe is unusually vascular. Except in the selachians, de Beer states, the pars intermedia is always very feebly vascularized. There are fairly numerous vessels in the pars tuberalis. The pars nervosa always contains a system of vessels just within its borders on the side of the pars intermedia but the rest of its substance is rather poor in vessels.

Stevens ('37) made quantitative measurements of the vessels in the hypophysis of the cat and reports that the volume of the vessels in a unit volume of tissue in the anterior lobe is six times as great as that in the posterior lobe though their relative lengths were 4:3. The capillaries in the latter lobe have the same diameter as in the brain proper ( $3.3\mu$ ) and their length per unit volume (1108 mm. per cubic millimeter) is comparable with that in the richest encephalic centers. The diameter of the vessels in the pars intermedia is also the same but their length is only about one-twelfth as great. The pars tuberalis has vessels even wider ( $7.6\mu$ ) than those of the pars anterior, while their length in a unit volume is comparable with that in the pars posterior.

The anatomy of the hypophysis of the frog has been well described by Atwell ('18) and by de Beer ('26) and no difficulty was experienced in recognizing and delimiting the four lobes as described by them, even in the material stained only with picric acid. The pars subdistalis described by Roofe ('37) in *Ambystoma* was not found to be distinct and was not considered in this study.

#### MATERIAL AND METHODS

Measurements were made in the hypophyses of ten leopard frogs. These animals had been kept under constant conditions in the vivarium of the department of biology from the end

of September, eight being prepared in October, the other two in March.

The frogs were anaesthetized with illuminating gas with amyl nitrite added and carmine gelatine was injected from the heart, after washing out the vessels with saline. Injection was commenced at a pressure of about 50 mm. of mercury (approximately normal blood pressure) and was raised gradually to various heights ranging from 100 mm. to 250 mm., this procedure being found necessary in order to procure complete filling of the vessels. The details for the individual specimens are recorded in table 1.

TABLE 1  
*Leopard frogs used*

| <i>Frog</i> | <i>Sex</i> | <i>Body length<br/>in millimeters</i> | <i>Date of<br/>preparation</i> | <i>Injection pres-<br/>sure in milli-<br/>meters Hg</i> |
|-------------|------------|---------------------------------------|--------------------------------|---------------------------------------------------------|
| 105         | ♂          | 68                                    | October 9                      | 100                                                     |
| 106         | ♀          | 65                                    | October 9                      | 50                                                      |
| 107         | ♂          | 63                                    | October 9                      | 210                                                     |
| 108         | ♀          | 70                                    | October 9                      | 150                                                     |
| 109         | ♀          | 72                                    | October 9                      | 240                                                     |
| 110         | ♀          | 62                                    | October 12                     | 185                                                     |
| 111         | ♂          | 64                                    | October 12                     | 210                                                     |
| 131         | ♂          | 72                                    | March 10                       | 150                                                     |
| 136         | ♀          | 64                                    | March 12                       | 250                                                     |
| 145         | ♀          | 85                                    | October 29                     | 200                                                     |

The brains and hypophyses were fixed in 10% commercial formalin, embedded in celloidin and cut into serial transverse sections 20  $\mu$  thick. In the earliest specimens, occasional sections were cut 100  $\mu$  thick, later every twenty-first section was so treated, and in the last cases every eleventh section was cut 200  $\mu$  thick. These thick sections were used to test the completeness of injection although it was realized that this provided only a slight check since they could not show what condition existed in the thin sections used for measurement.

Measurements of the vessels in a unit area of tissue were made by means of a square-ruled disc micrometer in each of ten successive sections, except in the case of the pars tuberalis. The latter was so small that the vessels in only a small



area were available and the measurements of all these areas in each specimen were added together (making a total volume of tissue in most cases less than half that used in the other lobes) and calculated to the same basis used in the other parts.

Different instruments were used by the two observers, in consequence of which the results were all reduced to the basis of a volume of tissue of  $100^3$  cu.  $\mu$  before being brought together.

In order to obtain a correction factor for shrinkage, the three dimensions of each of five brains were measured before fixation. After embedding, the excess celloidin was washed from the surface with ether alcohol and the measurements were repeated, revealing an average linear shrinkage of 10.7%. The small size of the hypophyses making it impracticable to determine directly the shrinkage of these organs in this way, the results thus obtained for the brain were employed in the present study. While this undoubtedly involves some error, the latter is probably smaller than that in the use of uncorrected values. It may be noted that Stevens ('37) found the shrinkage of the hypophysis of the cat to be fairly close to that of brain tissue in the rat.

It does not appear to be known whether or not active closing of capillaries takes place in the hypophysis. It is believed, however, that the higher pressures used in this case were sufficient to ensure the opening of most or all of the vessels and, as there is no correlation between injection pressure and the results of the measurements of vascularity, it is presumed that this was true in all the specimens.

#### *Diameters of the vessels*

The great width or sinusoid character of the vessels in the hypophysis or in its anterior lobe reported by various observers is mentioned above. Table 2 shows the results of the present measurements of diameter. These have been corrected for shrinkage on the assumption that the shrinkage of the vessels is equal to that of the brain tissue. The measurements in pars anterior and pars nervosa were made by both

TABLE 2

*Average diameters in micra of capillaries in hypophysis of leopard frog*

|                 | <i>On the slide</i> | <i>Corrected for shrinkage</i> |
|-----------------|---------------------|--------------------------------|
| Pars anterior   | 7.9                 | 8.8                            |
| Pars tuberalis  | 8.2                 | 9.2                            |
| Pars intermedia | 9.4                 | 10.5                           |
| Pars nervosa    | 9.0                 | 10.1                           |

authors, their results agreeing closely. The difference between the averages for these lobes was found to be thirteen times as great as its probable error. The appearance of the

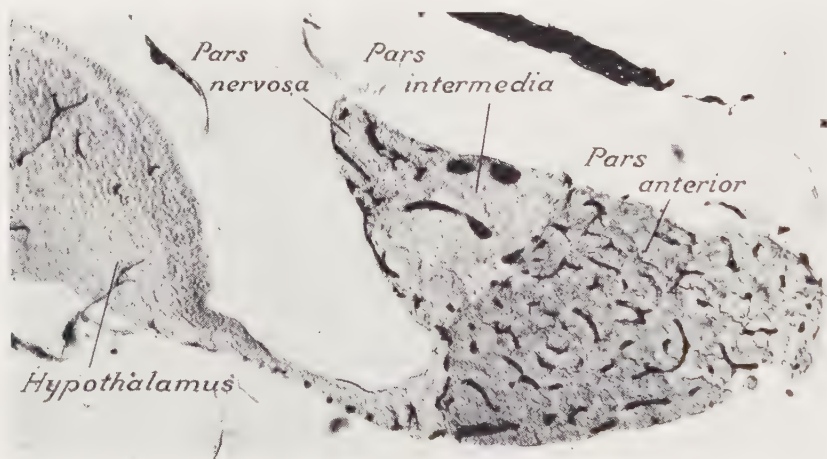


Fig. 1 Photomicrograph of sagittal section  $20\mu$  thick of hypophysis of *Rana pipiens*.  $\times 68$ .

vessels is shown in the accompanying photomicrographs (figs. 1 to 3).

It is evident that the vessels of the pars nervosa and those of the pars intermedia are much wider than those of the other two lobes. Even the latter, however, as may be seen in figure 1, are of markedly greater caliber than those in the brain (Craigie, '38 b), where the widest reported, in the hypoglossal nucleus, measured  $6.4\mu$  and the average diameter was  $5.6\mu$ . Thus the frog differs from mammals, where observers agree

that the capillaries in the pars nervosa have about the same diameter as those in the brain. The diameters are also notably greater than those recorded by Krogh ('29) for resting muscle in the frog, namely  $4.5\ \mu$ , or even for stimulated muscle ( $6.8\ \mu$ ).

The greater diameter of the vessels in pars nervosa and pars intermedia as compared with the anterior lobe is a point

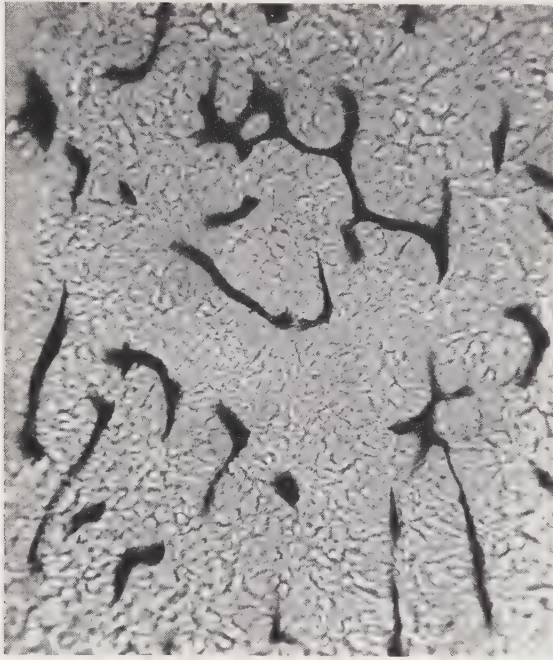


Fig.2 Photomicrograph of small part of transverse section of pars anterior of hypophysis of *Rana pipiens*.  $20\ \mu$ .  $\times 240$ .

of striking difference from conditions in mammals. Comparing the values with those recorded by Stevens ('37) for the cat, we find that those for the pars anterior and for the pars tuberalis are practically the same in these two species, but that for the pars anterior of the frog is two and a third times as great as that for the cat and that for the pars intermedia two and a half times as great.

De Vries ('34) believes that the capillaries in the brain are much wider in life than in mounted sections. He states, however, that while the caliber of the cerebral capillaries may be altered by the pressure used in injection, this is not the case with those of the hypophysis. In the present material, no



Fig. 3 Photomicrograph of small part of transverse section of hypophysis of *Rana pipiens*; pars nervosa above, pars intermedia below.  $20\ \mu$ .  $\times 240$ .

relation was found between observed diameters and injection pressure.

*Lengths of the vessels in a unit volume of tissue*

The length of the vessels in a unit volume of tissue was measured independently by each author in nine animals. Eight of the specimens were studied by both, while each examined one specimen not used by the other. Thus eighteen values, based upon ten specimens, were obtained for each lobe

of the hypophysis. Since different instruments were employed by the two observers, the unit volumes involved were different. Hence the results were all calculated to the basis of a cube of tissue of 100  $\mu$  edge and were then averaged, the values obtained being presented in table 3. At the top of each column the observer responsible is indicated by his initial. It will be seen that in the eight specimens examined by both, Taylor's measurements tend to be slightly the higher, though this is not so in every case. It would appear that either he tended to attribute slightly greater length to oblique or twisted pieces of capillary or else a certain amount of unconscious selection of areas to be measured took place. The personal factor, however, does not seriously affect the final result.

The fact that the probable error is so much larger in the pars intermedia than in the pars anterior or the pars nervosa may probably be attributed to the uneven distribution of the vessels in the pars intermedia, where they tend to occupy a peripheral situation, while the size of the error in the pars tuberalis may perhaps be due to the very small volume of tissue in which the measurements for this lobe had to be made. In this connection, however, it is of interest to note the statement of Rasmussen ('36) that in the human species, the "pars intermedia is probably the most variable structure in the body, both as to size and histological structure."

The values after correction for shrinkage are given in the first column of table 4.

The tables show that the vascularity in terms of length of the capillaries is greatest in the pars nervosa, not in the pars anterior in the frog, a condition which is at variance with those heretofore described elsewhere except in the case of Brown's ('24) observations on the rat, though confirming and extending de Beer's statement that the neural lobe of the frog is unusually vascular. After the pars nervosa, the pars anterior, pars tuberalis, and pars intermedia rank in that order in respect of capillary richness. Tilney's statement that the partes intermedia and tuberalis lack vessels and Atwell's finding that the pars tuberalis is non-vascular while the pars



TABLE 3

*Length in micra of capillaries in 100<sup>3</sup> cu.  $\mu$  of tissue in the hypophysis of Rana pipiens, not corrected for shrinkage*

| Frog                        | 105  | 106 | 106 | 107 | 107 | 108 | 108 | 108 | 109 | 109 | 110 | 110 | 111 | 111 | 131 | 131 | 136 | 136 | 145 | Aver- | P.E. |
|-----------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|------|
|                             | ♂    | ♀   | ♀   | ♂   | ♀   | ♀   | ♀   | ♀   | ♀   | ♀   | ♀   | ♂   | ♂   | ♂   | ♂   | ♂   | ♀   | ♀   | ♀   | age   | %    |
| Observer                    | T    | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   | T   | C   |       |      |
| Pars anterior<br>(distalis) | 736  | 594 | 546 | 787 | 659 | 693 | 559 | 667 | 481 | 605 | 604 | 656 | 595 | 739 | 605 | 689 | 648 | 626 | 638 | 638   | 1.9  |
| Pars tuberalis              | 313  | 389 | 292 | 583 | 522 | 312 | 207 | 382 | 316 | 229 | 179 | 256 | 335 | 354 | 288 | 283 | 301 | 225 | 320 | 320   | 5.1  |
| Pars intermedia             | 108  | 114 | 180 | 136 | 141 | 168 | 71  | 141 | 127 | 89  | 77  | 138 | 69  | 94  | 77  | 133 | 177 | 162 | 122 | 122   | 4.8  |
| Pars nervosa                | 1035 | 753 | 848 | 941 | 885 | 653 | 690 | 825 | 633 | 674 | 777 | 653 | 605 | 772 | 841 | 995 | 991 | 709 | 793 | 793   | 2.7  |

intermedia is fairly vascular in the frog are not confirmed, the former part being nearly three times as rich as the latter, though poorer than the pars anterior to a much greater degree than in the cat (Stevens, loc. cit.). Something of the relative richness can be seen by inspection of figures 1 to 3.

On the other hand, comparing the value for each lobe directly with the corresponding value given by Stevens for the cat, we find that the total length of the vessels in  $100^3$  cu.  $\mu$  of fresh anterior lobe tissue is only a little more than half as great in the frog as in the latter animal. The pars tuberalis of the cat is three times as richly vascular as that of the frog. The pars nervosa is only quite insignificantly richer in the cat, while the pars intermedia is richer in the frog.

TABLE 4

*Average length of capillaries in  $100^3$  cu.  $\mu$  of fresh tissue in the hypophysis of the leopard frog (coefficient for correction for shrinkage 0.80)*

|                 | <i>All specimens</i> | <i>Males</i> | <i>Females</i> |
|-----------------|----------------------|--------------|----------------|
| Pars anterior   | 510                  | 546          | 488            |
| Pars tuberalis  | 256                  | 303          | 226            |
| Pars intermedia | 97                   | 87           | 105            |
| Pars nervosa    | 634                  | 654          | 622            |

In the second and third columns of table 4 the lengths for males and females are presented separately, revealing a definitely greater vascular richness in the anterior lobe of the male than in that of the female. The neural and tuberal lobes also appear to be richer in the male but the probable errors in these are greater than in the pars anterior and the differences in them and in the pars intermedia are not statistically significant.

#### *Volumes and areas of the capillaries*

The caliber of the vessels in the different lobes being so different, the volume of the capillaries in a unit volume of tissue or the surface area of their walls may be more significant than their length alone, and these values have been calculated (after correction for shrinkage) and recorded in table 5.

The broad relations between the lobes in respect of these characters agree, of course, with the differences in capillary lengths. The proportional excess of the supply in pars nervosa over that elsewhere is, however, greater if measured in terms of volume or area, except as compared with the pars intermedia, in which case it remains about the same. The pars intermedia, while still much the poorest lobe of the hypophysis, does not fall so far behind the others when measured in these ways.

The volumes are all much greater than those in any part of the frog's brain examined (Craigie, '38 b) except in the case of the pars intermedia, which is considerably exceeded by the cochlear nucleus (the richest part of the brain). The

TABLE 5

*Volumes of capillaries and areas of their walls in the hypophysis of the leopard frog*

|                 | Volume in cu. $\mu$<br>of capillaries in<br>100 <sup>3</sup> cu. $\mu$ of<br>tissue | Surface area in<br>sq. $\mu$ of capillaries<br>in 100 <sup>3</sup> cu. $\mu$<br>of tissue | Area in sq. mm.<br>of wall covered<br>by 1 cu. mm.<br>of blood |
|-----------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|----------------------------------------------------------------|
| Pars anterior   | 31,043                                                                              | 14,750                                                                                    | 454.5                                                          |
| Pars tuberalis  | 17,016                                                                              | 7,398                                                                                     | 434.8                                                          |
| Pars intermedia | 8,609                                                                               | 3,250                                                                                     | 377.4                                                          |
| Pars nervosa    | 51,838                                                                              | 20,130                                                                                    | 392.2                                                          |

surface areas of the capillaries are less in both pars intermedia and pars tuberalis than in the cochlear nucleus, and the former is surpassed by the motor facial nucleus. These relations are shown in the accompanying graph (fig. 4).

On the other hand, the surface area to which a unit volume of blood is exposed (table 5, last column) is much less in all parts of the hypophysis than in any region of the brain, this character varying inversely as the radius of the vessels.

Again comparing the measurements in the hypophysis of the frog with those of Stevens in the corresponding parts in the cat, the surface area of the vessels in a unit volume of anterior lobe is only two-thirds as great as in the latter and in the pars tuberalis a little over one-third as great. In the neural lobe, however, it is nearly two and a half times as great,

and in the pars intermedia it approaches four times the extent in the cat.

In the pars anterior and the pars tuberalis the area of wall surface covered by 1 cu.mm. of blood is similar in the two animals compared, but in the other two lobes it is much less in the frog.

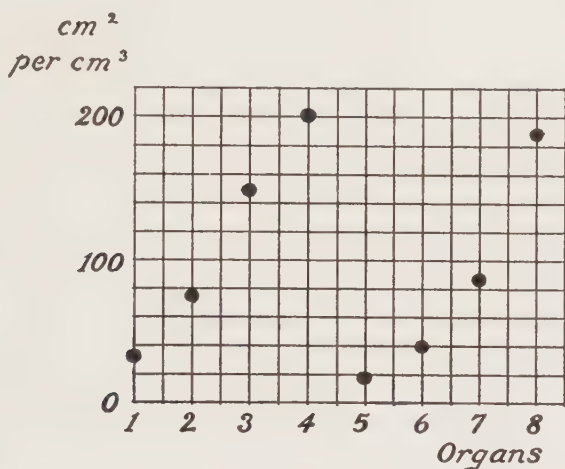


Fig. 4 Graph showing the relative surface areas of capillaries in a unit volume of tissue in the hypophysis, the brain, and skeletal muscle of the frog. 1, pars intermedia; 2, pars tuberalis; 3, pars anterior; 4, pars nervosa; 5, medial longitudinal bundle; 6, motor VII nucleus; 7, cochlear nucleus; 8, skeletal muscle (Krogh).

#### DISCUSSION

The foregoing comparisons between the measurements of the vascular supply in the hypophysis of the frog and those in the corresponding parts of the cat suggest that there must be important differences in hypophyseal physiology in these two animals. This would not be surprising since Smith ('35) states that there are marked differences even among different species of mammals both in "the pituitary content of at least certain of the pituitary hormones" and in response to these hormones.

The greater vascular richness of the pars anterior of the mammal is in keeping with de Beer's finding that "the anterior lobe gains in importance as one ascends the evolutionary scale." It cannot be linked simply with the more active metabolism of the warm-blooded animal, since two lobes of the hypophysis are more vascular in the frog than in the cat.

The pars anterior of the hypophysis of amphibians has been found to have an important part in growth regulation, probably acting indirectly through its effect on the thyroid gland (Smith, '16; Allen, '17; Adams, '33). It also probably helps to regulate sugar metabolism (Zwarenstein and Bosman, '32; Houssay, Benedetto and Mazzocco, '33; Collip, '35) and to maintain the potassium content of the serum (Zwarenstein, '33). Farther, the pars anterior was found by Houssay, Giusti and Lascano-Gonzalez ('29), by Shapiro and Zwarenstein ('33), and by Shapiro and Shapiro ('34) to be required for the maturation of the ova, an antagonistic effect being exerted probably by the pars nervosa, though Hahn ('12) states that gigantism and precocious development of the ovaries in tadpoles of *Rana esculenta* were associated with a hypophyseal hypertrophy which was greatest in the posterior lobe. The pars anterior also affects sexual activity in the male (Houssay and Lascano-Gonzalez, '29; Houssay, Giusti and Lascano-Gonzalez, '29; Rugh, '34).

The pars tuberalis keeps up the calcium content of the blood and affects color changes in the skin (Hasama, '31; Shapiro and Zwarenstein, '33, '34), the calcium-decreasing effect of hypophysectomy being more marked in the amphibian than in the mammal (Collip, '35). Antagonistic actions are exerted probably by the pars intermedia (Houssay and Giusti, '29).

Orías ('34) found the pituitary to be a factor in maintaining vascular tone in toads, the posterior lobe being mainly concerned.

These statements as to the physiology of the hypophysis and of its parts do not appear to offer much which can be linked with the observations reported in the present paper.



The only difference from mammals pointed out is the more marked importance of the pars tuberalis in maintaining blood calcium in the frog, yet the pars tuberalis is the part in which the cat most greatly surpasses the frog in vascular richness. Martins ('29) found evidence that the frog may possibly produce less than the mammal of the hormone stimulating ovarian activity, a fact which may be placed beside the poorer vascular supply of the pars anterior of the frog. The physiological significance of the richness and of the sinusoid character of the vascular supply of the partes nervosa and tuberalis is not suggested.

Krogh ('29, p. 30) has compared the surface areas of the capillaries in a unit volume of skeletal muscle in the frog and the dog and found the area in the latter to be three times as great as in the former. Comparing with his figure for skeletal muscle that for the pars anterior in table 5, we find that the ratio of the capillary surface in the pars anterior to that in skeletal muscle is  $\frac{148}{190}$  or 0.8 in the frog (fig. 4). Similarly comparing Stevens' value for the pars anterior of the cat with Krogh's for skeletal muscle of the dog we obtain a ratio of  $\frac{226}{590}$  or 0.4. These comparisons are, of course, crude, particularly the latter, which involves two mammalian species, but the difference between the two ratios is so great as to be almost certainly significant and suggests that the pars anterior may really be even more important to the frog than to the mammal. It may be remarked also that Krogh's value for frog muscle is based apparently on an assumed capillary diameter of  $15\ \mu$ , which is very much larger than his observed diameters (loc. cit., p. 65), so that if the latter were used the ratio of pars anterior would be still greater.

Sexual differences in respect of potency of hypophyseal extracts and of response to their action, as well as in histological structure, are known in mammals (e.g., Evans and Simpson, '29; Ellison, Campbell and Wolfe, '32; Rasmussen, '33 a, '33 b; McQueen-Williams, '35). The first two groups cited found male anterior lobe more potent than female in producing sexual precocity and stimulating ovulation in the

rat. Pfeiffer ('35, '36) stated that the male or female type of hypophysis is determined by the kind of gonad present before puberty in rats. Rugh ('34, '37) found that injection of macerated pituitary of *Rana pipiens* into the same or related species produced ovulation in females and amplexus in males, the pituitary of the female being twice as potent as that of the male, but this difference being quantitative only. These observations and the effect of anterior lobe secretion on the ovary demonstrated by Shapiro and Zwarenstein may be set alongside the sexual difference in vascularity found in the pars anterior, but the nature of the correlation between them, if it exists, is not clear.

In view of the apparent relationship between pars intermedia and chromatophore reactions, it may not be coincidence that a vascular pars intermedia occurs in the frog, where such reactions are important, while the corresponding organ is poorer in vessels in the cat, where they are of less significance. De Beer considers the pars intermedia to decrease in importance as the phylogenetic scale is ascended and it is reported to be absent in various higher vertebrates.

#### SUMMARY

The capillaries, or sinusoids, in all parts of the hypophysis of the frog are relatively very wide, the narrowest being those of the pars anterior, where the average diameter is  $8.8\mu$  as compared with an average diameter of the capillaries in the brain of  $5.6\mu$ . Those in the pars nervosa and in the pars intermedia are still wider, in contrast with the condition in mammals.

The caliber of vessels in the pars anterior and pars tuberalis is similar to that in the cat, but those in the pars nervosa are two and a third times as wide as in the latter animal.

In terms of length of capillaries in a unit volume, the pars nervosa is the richest division, followed in order by the pars anterior, the pars tuberalis, and the pars intermedia. The pars intermedia is richer in the frog than in the cat, the pars nervosa is about the same in both, and the other two lobes are much richer in the cat.

The pars anterior is more vascular in male frogs than in females, but the other lobes do not differ significantly in the sexes.

While the surface area covered by a unit volume of blood is much less in the hypophysis than in the brain, the volumes of the vessels in a unit of tissue are much greater except in the pars intermedia and the areas are greater in pars anterior and pars nervosa.

The surface areas in the pars nervosa and in the pars intermedia are much greater than in the cat and a larger volume of blood covers each unit of area.

The ratio of capillary surface in a unit volume of the pars anterior to that in skeletal muscle is apparently markedly greater in the frog than in the mammal.

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# THE TOPOGRAPHICAL ANATOMY OF THE SMOOTH MUSCLE OF THE CAT'S NICITATING MEMBRANE

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THREE FIGURES

In view of the extensive use of the cat's nictitating membrane for investigations of the behavior of smooth muscle, it is curious that neither physiologists nor anatomists have established clearly the exact position and extent of the smooth muscle involved. Conclusive physiological evidence of the existence of a smooth muscle retracting the cat's nictitating membrane dates back about 40 years (Lewandowsky, 1899). More recently this muscle has supplied physiologists with experimental data on which have been built important theories of the behavior of smooth muscle in general, the functions of the sympathetic nervous system, and the characteristics of neurohumors. None the less, physiologists seem to have no well-founded information as to the anatomy of the muscle, and a survey of the literature reveals, moreover, that the anatomical descriptions of the cat's nictitating membrane are relatively inadequate.

Indeed, many textbooks of comparative anatomy, including one of the most recent (Ihle et al., '27), deny that there is any smooth muscle in the mammalian nictitating membrane proper. More recently, however, Querido ('24), Zernik ('28), Stibbe ('28) and Lawrentjew and Borowskaja ('36) agree in finding a small amount of smooth muscle in the membrane of various mammals. Nevertheless, the amount of muscle found within the boundaries of the nictitating membrane

proper cannot account for the degree of retraction actually observed in the membrane. For in the cat it can be shown that the nictitating membrane is retracted by a force which can exert a tension of 4 or 5 gm., and which can move the mid-point of the free border of the membrane a distance as great as the expanse of the visible membrane itself. Consequently it would seem that most, although not all, of the smooth muscle which causes retraction of the nictitating membrane must lie outside the membrane proper, in some layer of the peribulbar tissue. The present study has been undertaken to show the extent and arrangement of the smooth muscle retracting the nictitating membrane of the cat. It is hoped that the results may create a sounder basis for the evaluation of certain physiological data.

#### MATERIAL AND METHODS

Serial sections  $20\mu$  thick were made of the entire orbital contents of two kittens after fixing with Bouin's fluid and embedding in celloidin. Van Gieson's stain (acid fuchsin and picric acid) was used to differentiate the smooth muscle from connective tissue. Other cats' orbits were dissected under low magnification after hardening in 10% formalin. The differentiation between smooth muscle and connective tissue was made in the dissected eyes by bathing them in a dilute solution of van Gieson's stain at appropriate stages of dissection. Smooth and striated muscle were discriminated by microscopic examination of sectioned material.

In the following description the terms superior, inferior, medial, or lateral will be referable to the four directions of a cross drawn in a frontal plane through the center of the cornea. Structures located in the four quadrants between these lines will be spoken of as lying in inferomedial, inferolateral, superomedial, or superolateral positions. In reference to the peribulbar layers, 'superficial' will apply to structures farther from the central axis of the eye, while 'deep' will mean nearer to the central axis.

## OBSERVATIONS

Before considering the relations of the smooth muscle supplying the nictitating membrane, a brief description of the topographical relationships of the membrane and of the main features of the orbital contents will be presented. The description can be followed by referring to the semidiagrammatic drawings 1 and 2.

The nictitating membrane of the cat is a crescentic fold of tissue covered with conjunctiva, lying between the eyelids and the eyeball. It has, therefore, a free border and two surfaces, designated as palpebral and bulbar, as well as a base defined by two fornices which are termed palpebral and bulbar. At its widest region, near the inferomedial radius of the cornea, the membrane is divided by a cartilage into two symmetrical parts, namely, an inferior portion ending in an inferior horn, and a superomedial portion ending in a superior horn. The cartilage is curved to fit the spherical surface of the cornea and sclera over which it glides. As pointed out by Stibbe ('28), it resembles a T-shaped tool for wiping windows, with an extensive edge along the free border of the membrane, adapted for wiping the cornea, and a handle directed posteriorly along the inferomedial surface of the eyeball. When the membrane is fully relaxed, the posterior tip of this handle and the well-developed harderian gland which envelops it are hidden behind the fornices of the membrane. These relationships can be seen in figures 1 and 2 in which the cartilage is outlined by a dotted line.

The orbital cavity may be considered to have the form of a cone into the open end of which has been put a sphere, the eyeball. This cone has an apex at the optic foramen and a base at the bony and fibrous anterior rim of the orbit. Its walls consist of the orbital fascia, a musculo-aponeurotic sheath, attached posteriorly around the optic foramen and the superior orbital fissure, and anteriorly around the rim of the orbit except in its lateral part, where, behind the lacrymal gland, it is continuous with the periosteum at the posterior edge of the orbital portion of the malar bone. In

the superior and inferior parts of the orbital fascia lies the orbital muscle, whose smooth muscle fibers are arranged circularly in reference to the central axis of the eyeball. In the figures the orbital fascia is represented as having been dissected away, excepting a cuff of it (OF) attached to the posterior pole of the orbit.

The orbital fascia lies immediately superficial to the layer which contains the nictitating membrane and its associated muscles. The orbital fascia can be dissected away fairly readily from the underlying structures, excepting medially where it encloses the belly of the superior oblique muscle (fig. 2, SO), adheres to the trochlear cartilage (T), and is attached to the inferior oblique muscle (IO) where the latter arises from the medial wall of the orbit. After separating the orbital fascia from these structures and reflecting it posteriorly, there is revealed a second fascial layer. The extent and relationships of this deeper layer, which contains the smooth muscles which retract the nictitating membrane, are shown in figures 1 and 2. This deep fascial layer arises from the sheaths of the rectus muscles of the eye near the middle of their bellies. It blends with the sheath of the levator muscle (LP) of the upper eyelid and the capsule of the lacrimal gland (L). It is attached to the trochlear cartilage (T) and gives sheaths to the two oblique muscles (SO, IO) as they pierce it. Anteriorly it passes into the eyelids (PI, PS) and into the nictitating membrane (NM). Two leashes of striated muscle from the external rectus muscle and the levator of the upper eyelid pass through it to attach in the

#### ABBREVIATIONS

|                                                   |                                      |
|---------------------------------------------------|--------------------------------------|
| A, inferior smooth muscle of nictitating membrane | LP, levator muscle of upper eyelid   |
| B, medial smooth muscle of nictitating membrane   | NM, nictitating membrane             |
| C, cornea                                         | OF, orbital fascia.                  |
| H, harderian gland                                | ORB, orbicularis oculi muscle        |
| IO, inferior oblique muscle                       | PI, inferior palpebral smooth muscle |
| L, lacrimal gland                                 | PS, superior palpebral smooth muscle |
| LE, lower eyelid                                  | S, sclera                            |
|                                                   | SO, superior oblique muscle          |
|                                                   | T, trochlear cartilage               |



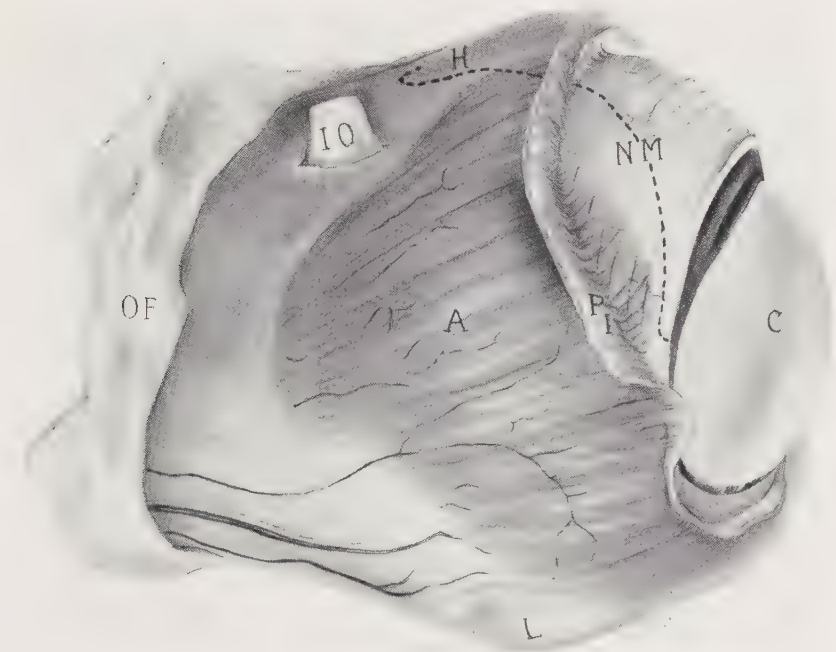


Fig.1 Semidiagrammatic drawing of the inferior aspect of the right orbit of kitten, dissected to show the relationships of the inferior smooth muscle (A) to the nictitating membrane (NM). The orbital fascia (OF) has been dissected free and reflected posteriorly. Anteriorly the cornea (C) is seen. The lacrimal (L) and Harderian (H) glands, as well as the origin of the inferior oblique muscle (IO), are shown. The dotted line indicates the edge of the cartilage of the nictitating membrane. The lower lid has been cut away close to its fornix. It can be seen how the inferior smooth muscle (A), arising extensively from the deep fascia of the orbit, passes forward to split into two laminae. The larger of these laminae passes into the lower eyelid to become continuous with the inferior palpebral smooth muscle (PI). The lesser sheet, formed by the splitting of the inferior smooth muscle (A), can be seen extending into the nictitating membrane. It will be observed that the muscle is restricted to a very small area of the nictitating membrane near the palpebral fornix. Consequently, the muscle fibers do not reach the border of the cartilage.  $\times \frac{1}{2}$ .

horns of the nictitating membrane. These cause protrusion of the membrane (Rosenbluth and Bard, '32). They are not shown in the drawings. This deep fascial layer also contains the smooth muscles (figs. 1 and 2, A, B) which cause retraction of the nictitating membrane and of the eyelids.

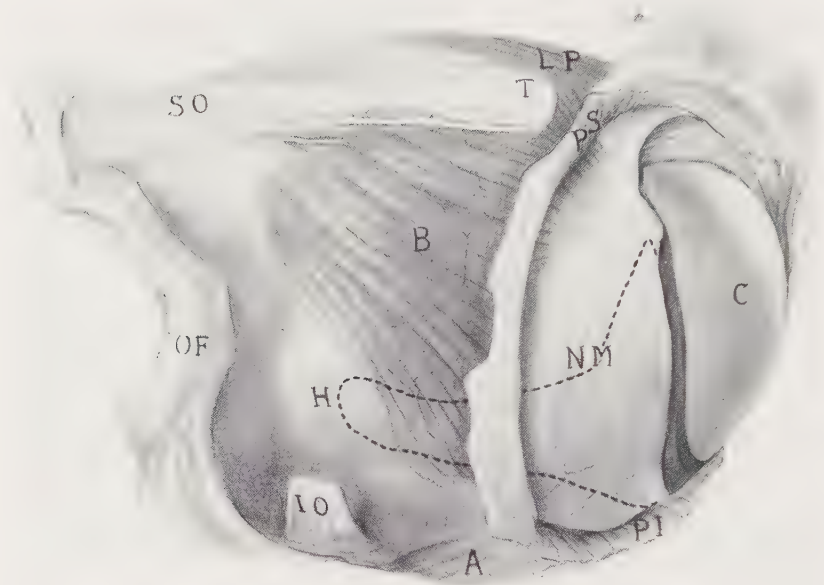


Fig. 2 The same kitten's orbit as in figure 1, rotated around the visual axis so as to show the medial aspect of the eye. This brings into view the medial smooth muscle (B) and shows its relationships to the nictitating membrane (NM). The harderian gland (H) and trochlear cartilage (T), as well as the superior oblique (SO) and inferior oblique (IO) muscles, are shown. The outline of the cartilage of the nictitating membrane is indicated by a dotted line. The eyelids have been cut away close to their fornices. It should be pointed out for further orientation of figures 1 and 2 that the inferior smooth muscle (A), as well as the palpebral smooth muscle of the lower eyelid (PI), is just visible at the lower edge of the dissected orbit shown in figure 2.

In figure 2 the medial smooth muscle (B) is seen taking origin in the deep fascia of the orbit. The muscle fibers pass forward to split into two portions, one passing into the upper lid, the other into the nictitating membrane. The sheet of smooth muscle fibers passing into the upper eyelid becomes continuous with the superior palpebral smooth muscle (PS), while the lesser sheet of muscle penetrates the nictitating membrane. The fibers of the medial smooth muscle, similarly to those of the inferior smooth muscle seen in figure 1, extend only a very short distance into the superomedial portion of the nictitating membrane. In consequence the nictitating membrane is in the main devoid of muscular tissue.  $\times \frac{1}{2}$ .

From this brief topographical introduction we shall now turn to a more detailed description of the smooth muscle related to the nictitating membrane. The smooth muscle which is associated with and retracts the nictitating membrane of the cat is arranged in two sheets less than 1 mm. thick which will here be designated as the inferior and medial smooth muscles. It will be seen later that these muscles correspond to the ones described by Groyer ('03), in the lion particularly, as the inferior and medial palpebral muscles. The position and extent of these two muscular sheets in the cat's orbit are shown in the two accompanying drawings. For convenience the inferior smooth muscle has been labelled 'A,' the medial one 'B.' These drawings will serve better than lengthy descriptions to familiarize the reader with the topography of the muscles and their relationship to the nictitating membrane. In each drawing the lids are shown cut away close to the fornix between the lids and the nictitating membrane. It is seen that the bulk of the inferior and medial smooth muscles lies outside the boundaries of the nictitating membrane in the deep layer of fascia described above. On approaching the nictitating membrane it is observed that each of the respective muscles splits into two components at the fornix between eyelid and nictitating membrane. The larger portion of each muscle enters the eyelid, while the lesser part inserts and ends in the nictitating membrane which it penetrates for a short distance.

The extensive line of origin of the inferior smooth muscle (A) from the deep fascial layer of the orbit can be seen in figure 1. It arises in an arc extending from the region of the lacrymal gland (L, below in the figure) to the neighborhood of the inferior oblique muscle (IO) close to its origin from the medial wall of the orbit. It arises in the main from the fascial sheath of the inferior rectus muscle, which, however, is not shown in the drawing because the deep fascia containing the inferior smooth muscle 'A' overlies and conceals it from view. From this origin the fibers of the inferior muscle are directed anteriorly across the surface of the inferior oblique

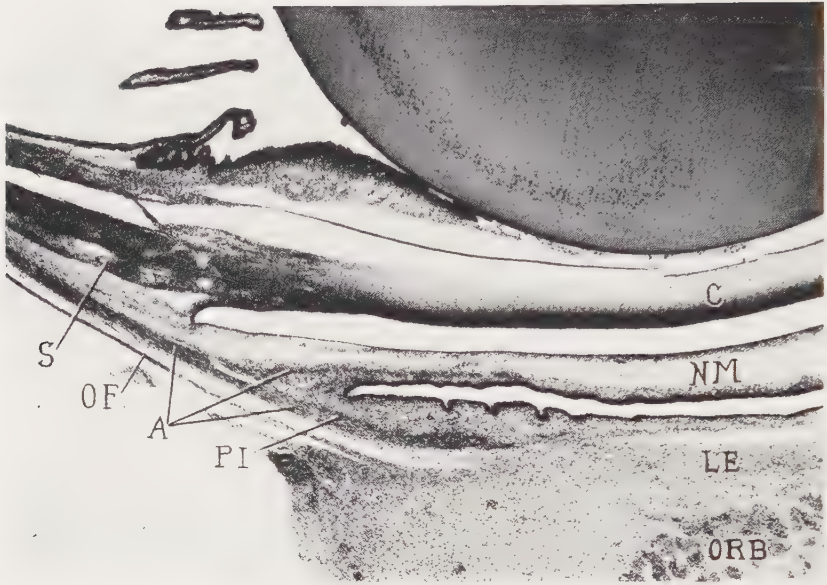


Fig. 3 Section of the orbital contents of kitten showing the relations of the inferior smooth muscle (A) to the nictitating membrane (NM). The smooth muscle is seen to lie between the orbital fascia (OF) and the sclera (S). At the palpebral fornix it divides into two laminae, one (PI) entering the lower eyelid (LE), the other penetrating the nictitating membrane (NM) for a short distance.  $\times 30$ .

muscle to insert into the lower lid (PI) and the inferior portion of the nictitating membrane (NM). The detailed manner in which the muscle splits is shown in figure 3. Here, one can see that just beneath the palpebral fornix of the nictitating membrane (NM) the inferior smooth muscle (A) divides into two laminae, one (PI) entering the lid close under the conjunctiva, the other entering the nictitating membrane close to its palpebral conjunctival surface. However, it can be seen, by referring to figure 1, that the fibers of smooth muscle which insert into the nictitating membrane extend in only a little way. They penetrate most extensively in the region of the inferior horn, but nevertheless they do not extend as far out even as the border of the cartilage. Returning to

figure 3, it will be observed that superficial to the inferior smooth muscle is the orbital fascia (OF), containing in this region the orbital smooth muscle whose fibers run in a circular direction at right angles to those of the inferior smooth muscle.

The medial smooth muscle (fig. 2, B) arises from the sheath of the body of the medial rectus muscle (which it conceals in the drawing) and from a stout attachment to the inferior and anterior edges of the surface of the trochlear cartilage (T). The fibers of the medial smooth muscle are directed anteriorly. The superior half of the muscle splits at the fornix between lid and nictitating membrane. As shown in figure 2, one portion of the fibers (PS) enters the upper lid, while the other portion inserts into the nictitating membrane. The lamina which enters the upper lid is continuous with the superior palpebral smooth muscle. The fibers of the inferior half of the medial smooth muscle reach the cartilage of the nictitating membrane and pass over the harderian gland (H), posterior to the palpebral fornix. The fibers skirting the harderian gland end near its inferior border in the deep fascia.

From these observations it can be concluded that the nictitating membrane of the cat is associated with two sheets of smooth muscle which lie in a layer of peribulbar fascia which is continuous with the superficial sheaths of the rectus muscles. One, here called the inferior muscle, arises from the fascia overlying the belly of the inferior rectus muscle and courses anteriorly, superficial to the inferior oblique muscle, finally splitting into two laminae, one of which enters the lower eyelid, one the nictitating membrane. The other muscle, here called the medial, arises from the fascia overlying the belly of the medial rectus muscle and from the trochlear cartilage. It courses anteriorly, finally also splitting, to insert in part into the nictitating membrane and in part to become continuous with the superior palpebral smooth muscle.



## DISCUSSION

A search of the literature shows that both peribulbar muscles demonstrated in our dissections have been previously described. Yet the manner and degree to which they penetrate the nictitating membrane, especially in the cat, have never been carefully studied. The literature has been concerned chiefly with the smooth muscle in the orbital fascia and in the eyelids. Attention has rarely been directed to two laminae of smooth muscle which are associated with the nictitating membrane and lie in the deep fascial layer.

Credit is generally given to Heinrich Müller (1872) for the first definitive and accurate description of the smooth muscles of the orbit. Nevertheless, a number of anatomists had previously seen these muscles in the gross. Thus, Albers (1802), Rosenthal (1811) and Rudolphi (1823) described muscular tissue connected with the nictitating membrane in the peribulbar region of several mammals. The orbital muscle proper was described in large mammals, such as the tiger, as early as 1811 by Rudolphi (1823). Some of this description was apparently accepted by writers of textbooks (Girard, 1820; Gurlt, 1834). Soon, however, the observations disappeared almost completely from the literature. In 1841, Bendz stated incorrectly that the orbital fascia is not muscular, but elastic. Excepting a reference in Siebold and Stannius' text in 1850, and a mention by Müller of Rudolphi's work on the tiger, the earlier work was almost forgotten.

From Müller's time to the present, references to the smooth muscle of the nictitating membrane have been meager, with the exception of that of Groyer. Müller concluded erroneously that movement of the nictitating membrane of mammals is controlled by a smooth muscle derived from the orbital muscle. Harling (1865) mentioned the smooth muscle of the membrane merely to disagree with Müller's statement regarding its origin. Subsequently Heinrich (1893) referred to it without deciding the question of its origin one way or the other. The more recent descriptions of the nictitating membrane by Querido ('24), Zernik ('28), Stibbe ('28) and

Lawrentjew and Borowskaja ('36) are none of them complete or fully accurate as regards its smooth muscle. The only description detailed enough to be useful is that by Groyer ('03). Studying the orbital contents of the lion and some other mammals, but not the cat, he found that there are two smooth muscles which attach to the nictitating membrane and which he designated as the inferior and medial palpebral muscles. Excepting certain differences set forth below, the smooth muscles described in the cat in the present paper conform to those reported by Groyer in certain other mammals.

A comparison of the present findings with the previous observations allows us to identify the inferior smooth muscle of the nictitating membrane in the cat with the inferior palpebral smooth muscle originally described by Müller (1872). Nevertheless, neither Müller, nor Heinrich (1893) and Zietzschmann ('04), who followed him, mention the connection of the inferior palpebral muscle with the nictitating membrane. Groyer ('03), on the contrary, working particularly on the lion, appears to have been the first investigator to establish such a connection. He did not, however, describe the exact limits of the muscle. From his description one gathers that the smooth muscle in the lion is more extensive than in the cat, for he speaks of the inferior palpebral muscle as crossing the harderian gland to join the medial smooth muscle. Moreover, according to him, the inferior palpebral muscle apparently encloses the inferior oblique muscle between two sheets of smooth muscle (Groyer, '03 and '06), instead of merely covering it as in the cat.

The medial smooth muscle of the nictitating membrane is less adequately described in the literature. There is nothing in the articles by Müller, Harling or Heinrich to show that they were acquainted with this muscle. Groyer is also the first author to present a clear description of and to define the medial muscle. Excepting the fact that the medial palpebral muscle of the lion joins the inferior smooth muscle, and his failure to state clearly the limits of the muscle in the nictitating membrane itself, his description coincides with ours in the cat.

Specific mention of the muscles associated with the nictitating membrane of the cat is to be found in the papers of Querido ('24), Stibbe ('28) and Zernik ('28). For Querido's fan-shaped muscle behind the harderian gland there appears to be no evidence. The muscle described briefly by Stibbe ('28) as extending backward toward the medial rectus is probably our medial smooth muscle. Zernik's ('28) description is limited to the distribution of muscle fibers within the nictitating membrane proper. He does not give an account of the arrangement of the fibers beyond the fornices of the membrane, in which region the principal masses of the muscles lie. We agree that in the membrane proper, as he points out, smooth muscle is restricted to the two horns. On the other hand, our evidence contradicts his belief that there are two distinct bundles of smooth muscle on each side, one a retractor and the other a protruder. We find, on the contrary, only one sheet of smooth muscle on each side which we regard as acting solely as a retractor. Indeed, by pulling forward the free border of the membrane in the partially dissected orbit (as in figs. 1 and 2), it can be seen that the direction of pull of the medial smooth muscle is medial, that of the inferior muscle inferior. The resultant direction of movement would be inferomedial, which is the actual direction of retraction of the membrane in the intact animal. Consequently the present findings appear to give some anatomical justification to the physiological evidence of Rosenblueth and Bard ('32) that the smooth muscle of the nictitating membrane is purely a retractor, whereas protrusion of the membrane is produced by leashes of striated muscle which are derived from the levator of the upper lid and from the lateral rectus muscle.

The present work gives no support to the assumption of Bacq and Monnier ('35) and of Eccles and Magladery ('37) that the smooth muscle in the nictitating membrane extends almost to the free border. And indeed Lawrentjew and Borowskaja ('36) had already stated briefly that the smooth muscle does not do so. We find that it does not pass far beyond the base of the membrane. Hence it seems unlikely

that 'injury' near the free border depolarizes smooth muscle cells, as Bacq and Monnier ('35) believe, and consequently it is unlikely that the electric potentials they record are demarcation potentials of smooth muscle as they hypothesize. For the same reason it seems improbable that the action potentials recorded by Eccles and Magladery ('37) between two leads placed on the membrane, as illustrated photographically in their paper, indicate conducted waves in smooth muscle between these two leads.

The limited extent to which smooth muscle exists in the nictitating membrane suggests also an explanation of why the membrane excised at its base fails to respond *in vitro* to stimulation. It should be mentioned, however, that preliminary experiments which we have carried out indicate that if the entire orbital contents be set up *in vitro*, the membrane will respond to adrenaline.

#### SUMMARY

This anatomical study was undertaken in view of the extensive use by physiologists of the smooth muscle retractor of the nictitating membrane of the cat and the prevailing ignorance of its topography.

The nictitating membrane of the cat is retracted by two smooth muscle sheets: 1) an inferior muscle, arising from the fascial sheath of the inferior rectus muscle and coursing anteriorly to the palpebral fornix of the nictitating membrane where it divides into two laminae, one of which enters the lower eyelid while the other enters the inferior part of the nictitating membrane; and 2) a medial muscle arising from the fascial sheet of the medial rectus muscle and coursing anteriorly to the papebral fornix of the membrane where it, too, divides into two laminae, one of which enters the upper eyelid while the other enters the superomedial part of the membrane. Neither muscle extends far into the membrane itself.



The inferior muscle corresponds to the inferior palpebral muscle originally described by H. Müller. Groyer described both of the muscles in the lion and other mammals, but not in the cat. Differences between the cat and the lion are pointed out.

Certain physiological conclusions based on false assumptions of the anatomy of these muscles are discussed.

The author is deeply grateful to Prof. George B. Wislocki for the privilege of working in his department and for his aid and interest in the work and to the other members of the department for their helpful suggestions.

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# AN UNUSUAL INSTANCE OF EOSINOPHIL PRODUCTION IN THE SPLEEN OF THE NEWT, *TRITURUS VIRIDESCENS*

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The urodele amphibia are characterized by a sharp segregation of erythrocytopoietic and granulocytopoietic tissues in different organs. Granulocytopoiesis as here used only refers to the production of eosinophils and neutrophils. In the newt and in Caudata generally the lymphogranulocytopoietic tissue occurs in the liver, predominantly in the subcapsular region, to a relatively very minor degree in the interstitial stroma. Erythrocytopoiesis occurs in the spleen. In the Proteidae the intertubular regions of the mesonephros constitute an important source of granulocytes in addition to the liver, and in the Plethodontidae (Barrett, '36) the bone marrow contains a small and variable amount of lymphogranulocytopoietic tissue. Thrombocytes are only produced in the erythrocyte-forming loci. Multiplication and maturation of both erythrocytes and granulocytes may be active in the general circulation, in the heart and in the hepatic and mesonephric sinusoids.

An exception must be specified as concerns the basophilic granulocytes; these granulocytes are produced in the spleen, to a small and variable degree in the intestinal mucosa.

No exception has been recorded heretofore to my knowledge to the apparently invariable rule in the case of the newt that eosinophils (and neutrophils) are produced only in the liver. Accordingly, an instance of extensive eosinophil formation in the spleen of this form presents a claim for investigation and brief report. The association of granulocytopoiesis with

erythrocytopoiesis in the spleen of one specimen of *Triturus* suggests at first consideration a reversion to hemocytopoietic conditions in lower vertebrates; e.g., cyclostomes and the African lungfish, where the primitive diffuse spleen constitutes the sole hemocytopoietic organ. This one instance demonstrates also the retention of an eosinophilopoietic potentiality in the spleen of a form in which this organ is normally erythrocytopoietic and non-granulocytopoietic.

In late June, 1937, fifteen apparently healthy specimens of *Triturus viridescens* were collected and placed in an aquarium in the laboratory. One was killed and tissues fixed in Zenker-formol on June 26th. Two were killed on July 1st. The remaining twelve either died or were killed during the following 2 weeks. Sections of the spleen of each of these fifteen specimens were microscopically examined. One of the two newts killed on July 1st (specimen C) had a spleen in which both erythrocyte and eosinophil formation were very active. Over a period of some 10 years prior to this experience I had examined sections of the spleen of at least fifty of these newts without noting any indication of granulocyte formation other than a very meager activity in basophil production. Between September 4, 1937, and January 17, 1938, I examined sections of the spleen of fifty additional specimens of *Triturus*, used in an experiment for the study of the effect of inanition on the blood cells. The evidence was definitely negative as concerned any activity in eosinophil production. This unique male specimen (C), in which the spleen showed extensive eosinophil formation, would seem to acquire special importance.

When this specimen was killed a supravital preparation of blood was made and studied. A notation made at the time, without recognizing its significance, was, 'many eosinophils.' As I now interpret this notation it shows that I was impressed by an apparently excessive number of eosinophils in the blood preparation. When I first saw a section of the spleen of this specimen my notation began to take on meaning. Accordingly, the condition of the perihepatic lymphogranulocytopoietic region of the liver required primary attention. This area,

however, was definitely normal; there was certainly no indication of hypoplasia.

I then examined sections of several regions of the intestine, mesonephros, testis and heart. The intestine contained various stages in the development of a trematode. The largest specimens of the parasite contained ova at various early stages of cleavage. Here presumably was the cause of the eosinophilia of this specimen of *Triturus*. The mucous layer of the intestine of this newt showed numerous patches of eosinophils; and a few of these granulocytes had migrated among the cells of the lining epithelium. The eosinophilia of itself was not remarkable in view of the intestinal parasitism; what seemed surprising and important was the fact that the stimulus for increased production of eosinophils did not evoke a hyperplasia of the lymphogranulocytopoietic tissue of the liver, but liberated a potential granulocytopoietic capacity in the spleen. Apparently the hepatic subcapsular and interstitial stroma was only capable of a certain limited normal activity in eosinophil production; additional demands were met by a response on the part of the spleen.

The primary point to emphasize is the fact that the splenic eosinophils did not to any unusual degree represent invasions from the blood stream. Many early stages in the maturation of eosinophils from lymphoid hemoblasts, in a very minor degree apparently from the stromal mesenchyme, could be seen. The appearance of the splenic areas of eosinophil production was practically identical with that of the perihepatic lymphogranulocytopoietic tissue. Rather unexpectedly, however, in neither area could eosinophils be found in process of proliferation.

The spleen of specimen (C) was comparatively large and essentially lymphoid. It contained a few typical lymphoid nodules, with medium-sized and small lymphocytes predominating. Certain of the nodules had a germinal center of principally larger lymphocytes, some in mitosis. The sinuses were well filled with erythrocytes in every phase of maturation, from hemocytyoblasts (larger lymphocytes) to normoblasts. Many cells of all these stages were in mitosis. Mitosis



was especially frequent in the erythroblast stage. Stages in the differentiation of thrombocytes from small lymphocytes also appeared in the venous sinuses. There were comparatively few macrophages in this spleen, and only scattered pigment.

The most striking feature of this spleen concerns the great abundance of eosinophilic granulocytes, especially in the subcapsular region. The presence of such large numbers of eosinophils at various stages of differentiation in this spleen should give a favorable opportunity for the detection of a possible genetic relation between basophils and eosinophils. The restriction of basophil production to the spleen in *Rana pipiens*, *Triturus viridescens*, *Proteus anguineus* and *Plethodon cinereus* was previously reported. A study of the basophils of the spleens of these amphibians led to the conclusion that these cells represented in part immature, in part abortive eosinophils. The basophils interpreted as immature eosinophils were small cells with lymphocyte-like nucleus. Stained with eosin-azure the granules of these cells were blue. The granules of the basophils interpreted as abortive eosinophils stained a deep purple. The granules of the 'immature eosinophils' were of spheric shape and of approximately uniform size; those of the 'abortive eosinophils' were generally of angular form and non-uniform size.

The eosinophilic spleen of the newt (C) contained the usual number and varieties of basophilic granulocytes. They clearly arose from lymphoid hemoblasts, in every morphologic feature similar to those which served as ancestors for the eosinophils. Moreover, they generally occurred in very widely scattered groups of three or four, as if very specific local conditions might have determined their origin, in contrast with adjacent more numerous eosinophils. In specimens of marrow stained with eosin-azure from pigeon and chicken and in the thymus of the turtle (Jordan and Looper, '28), the origin of eosinophils with bacillary granules (heterophils) from lymphoid hemoblasts with spheric basophilic granules could be traced through a closely graded series of maturation stages, an

intermediate 'hybrid' stage containing both basophilic and eosinophilic spheric granules in variable proportions. Clearly in these cases eosinophilia of granules was the result of a ripening process in originally basophilic granules. At a certain early brief period these eosinophils of the chicken were oxydase-positive as revealed in marrow spreads counterstained with eosin-azure.

However, in the case of the spleen of *Triturus* (C) no such transitional stages occurred between basophils and eosinophils. Moreover, the lymphogranulocytopoietic subcapsular envelope of the liver, where eosinophil production is also active, lacked completely any variety of basophilic granulocyte. No evidence accrues here in support of the apparently well-established conclusion that the basophilic granulocytes of the blood represent immature and abortive eosinophils. The granules of the eosinophils of the newt do not pass through an immature basophilic phase; they are eosinophilic and oxydase-positive from the beginning.

Obviously the spleen of *Triturus*, and presumably of other urodeles, retains the capacity for direct eosinophil production. Occasionally under certain conditions, as in the instance of parasitized specimen (C), this potential capacity may express itself to a considerable degree. But since granulocytopoiesis has been shifted in general to the liver in the phylogenetic group immediately above the fishes, it may be inferred that under normal conditions the hepatic subcapsular stroma is a more favorable region in urodeles than the spleen for eosinophil production. On this basis may be supported the concept that the comparatively meager granulocytopoietic activity expressed under the presumably less favorable conditions of splenic histology results in abnormal or abortive eosinophils, namely, basophilic granulocytes. The fact that the granules of the basophils, in contrast with those of the eosinophils and the neutrophils, are oxydase-negative is in accord with such interpretation.

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# MICROSCOPIC CHANGES IN THE PANCREATIC GLAND OF THE CAT PRODUCED BY SYM- PATHETIC AND PARASYMPATHETIC STIMULATION

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TWO PLATES (ELEVEN FIGURES)

A combined physiological and histological investigation of the pancreatic gland of the cat was undertaken with the object of studying some of the effects produced by the action of the sympathetic and parasympathetic secretory nerves and also of certain sympatho- and parasympathomimetic drugs. The histological part of the work was confined to study of the discharge of the zymogen granules from the acinous cells when the gland was stimulated to secretory activity by various methods. The more apparent changes in the islands of Langerhans were also noted.

A similar investigation of the histological changes in the acinous cells of the dog's pancreas was previously performed by Babkin, Rubashkin and Savitch ('09). However, the conditions regulating pancreatic secretion in the cat are somewhat different from those obtaining in the dog, especially as regards the effect of vagal stimulation. In the dog vagal stimulation elicits a small amount of pancreatic secretion, but in decerebrate cats it has been found impossible to obtain a visible secretion of pancreatic juice under prolonged rhythmic stimulation of the vagus nerves or under pilocarpine (Korovitsky, '23). Hence it was thought desirable to study the above-mentioned changes in the pancreatic gland of the cat, including the more apparent changes in the islands of Langerhans.

## METHODS

*Physiological methods*

The cats were fasted for 24 hours before experiment. During brief ether anesthesia a mixture of urethane and chloralose (0.5 gm. and 0.05 gm. respectively per kilogram body weight) was given intravenously. Tracheotomy was then performed, and the vagus nerves were cut in the neck and placed on electrodes. The stomach and duodenum were separated by tying of the mucous and submucous membranes, the muscular layer being left intact. When necessary, the pancreatic duct was cannulated, and a cannula was inserted also into the duodenum (in the latter case, in order to introduce HCl). In one control experiment, the spinal cord was cut below the medulla during brief ether anesthesia, artificial respiration was applied, and the vagus nerves were stimulated. In experiments involving splanchnic stimulation, the splanchnic nerves were tightly ligatured and placed on shield electrodes, and the blood pressure was recorded in order to test the efficacy of each stimulation. In some of these experiments, the suprarenal glands were removed.

The vagus or the splanchnic nerves were stimulated rhythmically with an induction current. In either case the nerves were stimulated alternately for 5-minute periods, with rest intervals of 2 or 3 minutes, the stimulations being continued for  $1\frac{1}{2}$  to 6 hours. During this time the current was gradually increased from 14 cm. to 7 or 8 cm. Adrenalin (Parke, Davis & Company's) and choline chloride solution, when used as stimuli, were introduced intravenously.

*Histological methods*

At the beginning of each experiment a control sample of tissue was taken from the pancreas and placed in freshly prepared Zenker-formol solution. Latterly a modified form of this solution (Baley, '37) was found to be more suitable, viz.:

|                                               |        |
|-----------------------------------------------|--------|
| 3% mercuric chloride                          | 45 cc. |
| 3% potassium bichromate                       | 45 cc. |
| 40% formalin (added immediately before using) | 10 cc. |



At intervals during the experiment pieces of pancreas were excised and placed in this solution, and at the conclusion of the experiment the gland was fixed by intra-arterial injection of the same solution. The tissue was embedded in paraffin. Sections were cut, 4 or 5  $\mu$  thick, and stained with neutral gentian (crystal-violet-orange G). For control purposes, two cats, which had been fasted for 24 hours, were anesthetized with nembutal and then injected intra-arterially with Zenker-formol solution. Similarly two cats, which had been fed 3 hours before, were anesthetized with nembutal and injected with the fixing fluid.

#### EXPERIMENTAL RESULTS

##### *The resting gland*

The acinous cells of the pancreatic gland of a cat, fasted for 24 hours, on microscope examination are seen to be in a quiescent state. The cells are filled with zymogen granules, which, under neutral gentian staining, show up deep violet against a background of pale grayish-violet cytoplasm. The ducts are empty. The transparent areas of the islands of Langerhans stand out in marked contrast to the acinous tissue; only the  $\beta$ -cell cords are visible, their granules being stained deep blue. Most of the islands are in very close contact with the acinous tissue; there appears to be no capsule separating them.

The acinous cells in the immediate vicinity of the islands are more densely packed with zymogen granules than are those in the more remote parts of the lobule, so that a kind of peri-insular circle, or 'halo,' of unexhausted acinous cells appears around each island. This phenomenon was formerly observed by Jarotsky (1899), Dale ('05), Schafer ('20), and Dolley ('25). (Figs. 1 and 2.)

When in a fasting animal it happened that the pancreatic gland had been in a state of moderate secretory activity at the beginning of the experiment, owing to the presence of some food residues in the stomach, the acinous cells surrounding the islands and forming the 'halos' were more distinctly

visible than in the quiescent gland. In these moderately active glands, secretion could also be seen in the ducts; when there were no food residues in the stomach or intestine the pancreatic ducts were always empty. In animals fed 3 hours before experiment, the microscopic picture was similar to the above, though the changes were more pronounced; the 'halos' were more numerous and more strongly marked, and the ducts were in many instances filled with secretion.

#### *Stimulation with hydrochloric acid*

Introduction into the duodenum of about 10 cc. of 0.2% HCl at intervals of  $\frac{1}{2}$  hour, during a period of 3 to 5 hours, elicited a flow of pancreatic juice, the volume of which varied in different experiments. In sections of pancreas taken from animals so treated, it was observed that most of the acinous cells were well filled with zymogen granules. The granules occupied the apical zone of the cells, but were not so closely packed as after vagal stimulation (see below). They were somewhat smaller than the granules in resting cells. The ductules were practically free from stainable material, so that, as they had probably contained some secretion, this must have been of a watery consistency. The ductules were not dilated—as is to be expected when a watery secretion, which escapes rapidly, is being produced (fig. 3).

#### *Vagal stimulation*

The vagus nerves were stimulated rhythmically in the neck with an induction current (coil 14 to 7 cm.) for a period of  $1\frac{1}{2}$  to 6 hours. Small samples of pancreas for histological examination were taken at intervals during the experiment. In some of the experiments the pancreatic duct was cannulated, but it was a striking fact that, in spite of strong and prolonged stimulation of the vagus nerves, there was practically no visible secretion of pancreatic juice. One control experiment was performed in which, in order to avoid the possible unfavorable effect of anesthetics on the functioning

of the pancreas, the spinal cord had been cut below the medulla and all the blood vessels going to the head tied. Although the effect of the small amount of ether used at the outset undoubtedly soon passed off, the effect of vagal stimulation in this experiment was also practically negative. Thus, during  $4\frac{1}{2}$  hours of vagal stimulation the gland secreted only 0.4 cc. of juice, whereas the introduction of 25 cc. of 10/N HCl into the duodenum provoked a flow of juice amounting to 1.6 cc. in 23 minutes.

Notwithstanding the absence of a flow of juice, prolonged vagal stimulation produced extremely great morphological changes in the gland (figs. 4 and 5). Most of the acinous cells were found to have become entirely depleted of their zymogen granules. Where zymogen granules still remained in the cells, it was observed that they were congregated in the apical region. The cytoplasm of the acinous cells had become yellow, the nuclei being only faintly visible. The ducts were filled with material which took the same stain as the granules, and they had the appearance of a tracery of branches and twigs. About 70% of all the islands, as seen under low power magnification, were surrounded with 'halos.' However, in the acinous cells forming these 'halos' the granules were much less numerous than in the cells of the resting gland. The blood vessels were dilated and filled with erythrocytes. If the period of vagal stimulation was shorter, the above-described changes in the gland were less pronounced.

In later stages of vagal stimulation the islands underwent marked changes. The outlines of the cell cords had become indistinct, vacuoles had appeared in them, and the capillaries were dilated and filled with red blood corpuscles. Some of the islands appeared to be greatly distorted. In many places between the islands and the acinous tissue, groups of cells could be discerned which in their apical part contained some zymogen granules and in their basal part were stained the same blue color as the granules of the  $\beta$ -cells (fig. 6).

*Stimulation with choline*

Choline chloride in equal doses of 2.5 mg. to 5 mg. was introduced intravenously at intervals of 5 or 10 minutes during a period of 2 to 4 hours (a total of 160 mg. to 337 mg. of choline being administered). When the pancreatic gland is stimulated with choline, the histological picture is similar to that obtained when the gland is stimulated through the vagus nerves. Under low power magnification, 80 to 90% of the islands are seen to be encircled by 'halos' (fig. 7).

*Stimulation of the splanchnic nerves with the suprarenal glands intact*

Rhythmic stimulation of the splanchnic nerves with an induction current (coil 14 to 7 cm.) during a period of 3 to 6 hours also produced changes in the acinous and the island tissue, although no secretion appeared in the cannula which had been introduced into the pancreatic duct. These changes, however, were less drastic than those which resulted from vagal stimulation. The zymogen granules had been partly discharged from the cells and the secretion could be seen in the ducts. The capillaries contained many erythrocytes and were dilated, but less so than after vagal stimulation. It could be observed that about 40 to 50% of the islands were encircled by 'halos.' Certain changes had occurred in the islands themselves. The cell cords showed many irregularities and contained vacuoles. In many places they seemed to be continuous with the acinous tissue, especially in the experiments where there was prolonged stimulation (figs. 8 and 9). The capillary network was dilated and contained red blood corpuscles.

*Stimulation of the splanchnic nerves after removal of the suprarenal glands*

After the suprarenals had been removed, the histological picture obtained when the pancreatic gland was subjected to the effect of rhythmic splanchnic stimulation during a period

of  $1\frac{1}{2}$  to 5 hours resembled that described in the previous section, but the changes were much more pronounced (fig. 10). The acinous cells in the center of the lobule, particularly around the islands, were depleted of zymogen granules; those at the periphery of the lobule contained fair numbers of granules. There was secretory material in the ducts. 'Halos' of unexhausted acinous cells round the islands were rarely present. In this type of experiment the 'halos' had begun to disappear very early, i.e., at the end of the first  $1\frac{1}{2}$  to 2 hours of splanchnic stimulation. The islands themselves were hardly visible under low magnification, appearing as transparent areas. Under high power these areas were seen to contain colorless cords of island cells and dilated capillaries, and the island cells were in very close contact with the surrounding acinous tissue. Whatever explanation may be given in regard to all these changes to be observed in the insular cells, it is clear that, with the suprarenals removed, even brief stimulation of the splanchnic nerves causes profound modifications to occur.

#### *Stimulation with adrenalin*

In response to intravenous injection of various amounts of adrenalin no visible secretion emanated from a cannula inserted into the pancreatic duct, nor was there any marked discharge of zymogen granules from the acinous cells. Adrenalin solution was injected during a period of 4 to 5 hours, samples of the pancreatic tissue being taken for histological investigation at intervals during the experiment. Massive doses of adrenalin corresponded to 1 cc. of 1:10,000 solution administered every 5 minutes. Less intense stimulation of the gland was produced by the injection of adrenalin solution of the same concentration at 15-minute intervals. The impression obtained from these experiments was that during the first  $1\frac{1}{2}$  to 2 hours of adrenalin administration there was a slight diminution in the numbers of granules in the acinous cells. Subsequently up to the fourth or fifth hour of adrenalin administration there was no further diminution and all the



acinous cells still held fair quantities of granules (fig. 11). Thus the 'halos' do not stand out so distinctly as in glands that have been subjected to other types of stimulation. The islands are clearly discernible on account of the deep blue granules of the  $\beta$ -cells, and have the appearance of being somewhat hypertrophied. It has been found by other investigators that prolonged treatment of animals with adrenalin (injected over periods of up to 30 days) produced great changes in most of the organs and in many instances resulted in degenerative processes setting in. The data concerning the effect produced on the islands by prolonged adrenalin administration are, however, conflicting. Satwornitzkaia, Simnitzky and Spassky ('32) and Bernabeo ('35) noted hyperplastic phenomena in the island cells; Igura ('27) found the islands to be atrophied, and Samson ('32) described them as normal.

#### DISCUSSION

The cat has proved to be an exceptionally suitable animal in which to study the more intimate mechanism regulating the secretory activity of the pancreatic gland. Whereas in the dog stimulation of the vagus nerves produces a definite though scanty secretion of juice rich in organic material, in our experiments on the cat only 0.25 to 0.5 cc. of secretion could be obtained from the pancreatic duct during 4 or 5 hours of vagal stimulation. Nevertheless the microscopic changes in the cells of the cat's pancreas under these circumstances were as great as in those of the dog's. The large zymogen granules had disappeared almost entirely from the acinous cells and small ones remained, a condition which would indicate that, under the influence of the vagus, certain intimate processes occurred in the acinous cells which changed the appearance of the secretory material. Many of the acinous cells were entirely depleted of their granules, and where granules still remained these had accumulated in the apical region of the cell. The ductules were filled with material stainable with the same dye as the granules. Thus we have here an exceptionally good illustration of the type of nerve effect which

Heidenhain (1878) designated 'trophic.' Since the organic colloidal material was evacuated from the acinous cells probably quite unaccompanied by water, it remained in the ductules, where it was coagulated by the fixing reagents. Therefore in the cat the vagus supply to the pancreatic acinous tissue is purely 'trophic.' Again the humoral stimulus (i.e., secretin, formed in the duodenum in response to HCl administration) produces what Heidenhain termed a 'secretory' effect. Under its influence water and salts pass through the cells in abundance, only a small amount of organic colloidal material being discharged from them. As already observed by previous investigators, the pancreatic juice under such circumstances is poor in organic material and enzymes.

Stimulation of the vagus nerves seems also to produce another effect on the acinous tissue. Mention has already been made several times under 'Experimental results' of 'halos' of unexhausted acinous cells, surrounding the islands and containing fair numbers of secretory granules. These were observed in the pancreas of control animals and of animals that had recently been fed, or had received HCl. The presence of these 'halos' is one of the most characteristic histological effects of prolonged vagal stimulation or of choline administration. On the other hand, a diminution in the number of 'halos' is in most cases characteristic of the histological picture following splanchnic stimulation, especially when the suprarenal glands have been removed. No definite explanation of this phenomenon can now be offered. However, an attractive hypothesis would be that the acinous tissue in the close vicinity of the islands is subjected to the action of insulin diffused from the islands. Miss Hebb ('37), working in our laboratory, has shown that insulin inhibits the activity of the acinous cells and diminishes, or even reverses, the action of the vagus nerve. There are good grounds for believing that the vagus is a secretory nerve for the islands (compare La Barre, '33). Therefore, when the vagus is stimulated, its 'trophic' effect might be diminished in those cells which are subjected to the immediate action of the insulin

secreted by the islands. Selye ('37) observed that in the pancreas of rats, in which an 'alarm reaction' had been produced by the administration of adrenalin, formaldehyde or morphine sulfate, the acinous cells in the immediate vicinity of the islands retained their secretory granules much longer than those in the regions more remote from them. In the present investigation peri-insular 'halos' were observed in the pancreas of control animals and were diminished or increased according to the type of stimulation to which the gland was subjected, but it is very doubtful that they were the result of the general, unspecific traumatic stimulus of an 'alarm reaction.' In special control experiments on cats, which were subjected to the usual preliminary operative procedure here employed (the vagi being cut in the neck and the pylorus ligatured, etc.) but were not further interfered with, no diminution in the number of zymogen granules in the acinous cells of the pancreas was noticeable; the 'halos' were no more marked in samples of pancreas obtained 4 hours after the operation than in the control pieces.

The different effects produced on the pancreas by splanchnic stimulation (with the suprarenal glands either intact or removed) and when adrenalin was administered is probably attributable to the antagonistic effects of the sympathetic nerve and adrenalin on the acinous cells. Whereas splanchnic stimulation with the suprarenals intact, and more especially with the suprarenals removed, causes a discharge of zymogen granules (as does vagal stimulation), administration of adrenalin seems to inhibit this entirely. Since an intimate relationship exists between carbohydrate metabolism and the secretory activity of the pancreatic gland (Still, Bennett and Scott, '33; Hebb, '35, '37, '38), the discrepancy between the effects of splanchnic nerve stimulation and adrenalin administration might perhaps be accounted for by the peculiar effect which adrenalin exerts on carbohydrate metabolism. According to Cori ('31) the carbohydrate metabolism is eventually inhibited by adrenalin, which diminishes the utilization of sugar in the tissues.

The changes brought about by vagal and splanchnic stimulation respectively in the histological picture of the pancreatic gland are not entirely similar. The presence of 'halos' of unexhausted acinous cells around the islands of Langerhans was found to be very characteristic of the vagus effect, as well as of choline administration. A decrease in the number of 'halos' was a typical phenomenon in the splanchnic experiments. This difference in the effects of the vagus and the splanchnic nerve may perhaps be explained by the theory that the island tissue is acted upon by the vagus but not by the splanchnic. The histological studies of De Castro ('23) on the mouse's pancreas lend support to this theory. He came to the conclusion that the vagus innervates the acinous tissue of the pancreas and also the islands of Langerhans, while the sympathetic nerve fibers supply only the blood vessels to the gland. If this view is accepted, then it is clear why the stimulation of the splanchnic nerves does not increase the output of insulin, which would check the discharge of zymogen granules from the near-by acinous cells.

#### SUMMARY

1. Prolonged stimulation with an induction current of the vagus nerves in the cat did not elicit any visible secretion which could be collected from the cannulated main duct of the pancreatic gland.

2. Microscopic investigation of the pancreatic gland, after it had been subjected to prolonged action of the vagus nerves, showed that a) the acinous cells were almost entirely depleted of secretory granules, and b) the small ducts were distended with a material stainable with the same dyes as the granules.

3. The introduction of a solution of HCl into the duodenum produced a flow of pancreatic juice, but histological examination showed that the discharge of secretory granules from the acinous cells was very slight.

4. Intravenous injection of choline chloride produced changes in the pancreatic gland similar to those brought about by vagal stimulation.

5. There was no discharge of secretory granules from the cells of the pancreas in response to intravenous administration of moderate or of massive doses of adrenalin.

6. The effect of splanchnic stimulation on the acinous tissue of the pancreas was similar to that produced by vagal stimulation; it was much more pronounced when the suprarenal glands had been removed than when they were left intact.

7. Under vagal stimulation or choline administration the discharge of granules from the acinous cells surrounding the islands of Langerhans was apparently slower than from the cells in the other parts of the pancreatic lobules, as shown by the presence of 'halos' of unexhausted acinous cells around many of the islands.

8. Changes were observed in the structure of the island tissue as a result of stimulation of the secretory nerves of the pancreas as well as after administration of choline.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Pancreas of normal cat (fasted 24 hours). The 'halos' of acinous cells containing undischarged zymogen granules stand out distinctly around the islands of Langerhans.  $\times 75$ .

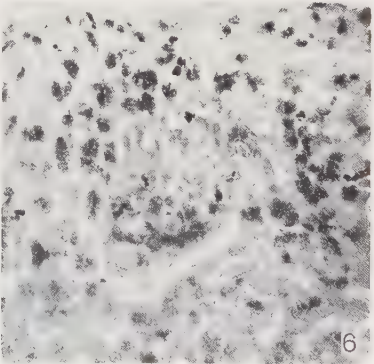
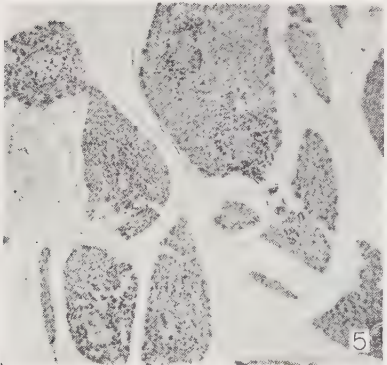
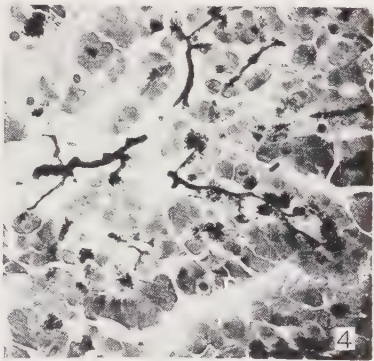
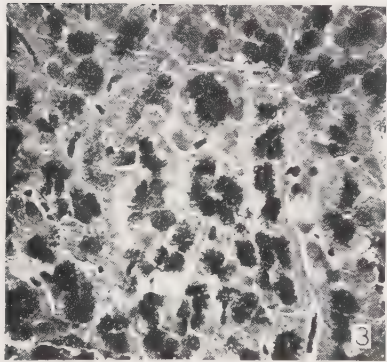
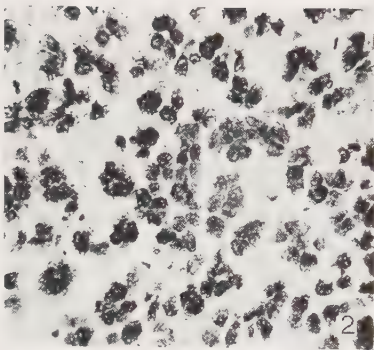
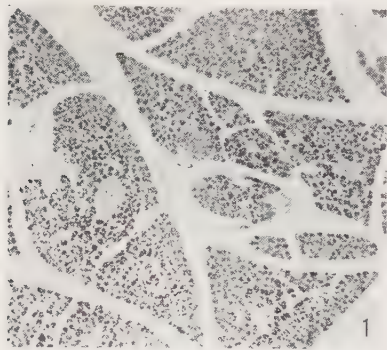
2 Pancreas of normal cat (fasted 24 hours). The acinous cells are filled with zymogen granules.  $\times 400$ .

3 Effect of stimulation with HCl. The numbers of zymogen granules in the acinous cells are not appreciably diminished. No secretion is visible in the ducts.  $\times 400$ .

4 Effect of vagal stimulation. Secretion may be seen in the ducts. The numbers of granules in the cells are much diminished; some of the cells are almost empty.  $\times 400$ .

5 Effect of vagal stimulation. With the exception of those forming 'halos' around the islands, the acinous cells contain practically no granules.  $\times 75$ .

6 Effect of vagal stimulation. One of the islands. Note the close contact between the island and the acinous tissue. The island is distorted; it contains a number of vacuoles, and many erythrocytes are present in the capillaries.  $\times 300$ .



## PLATE 2

### EXPLANATION OF FIGURES

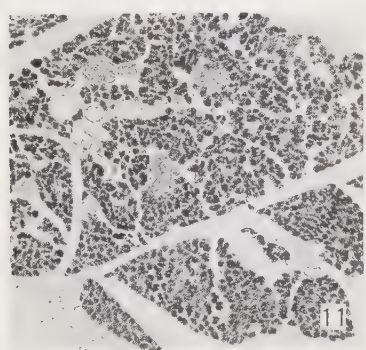
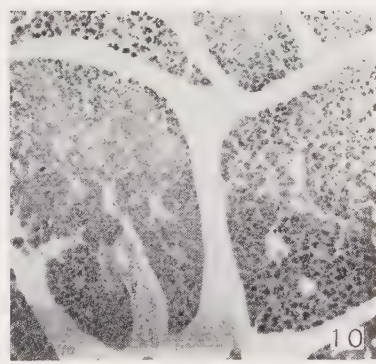
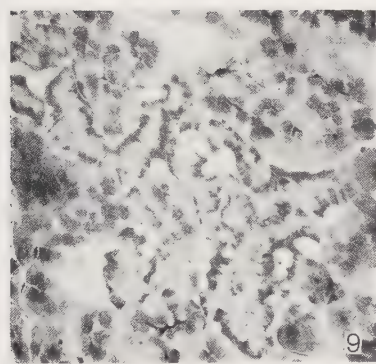
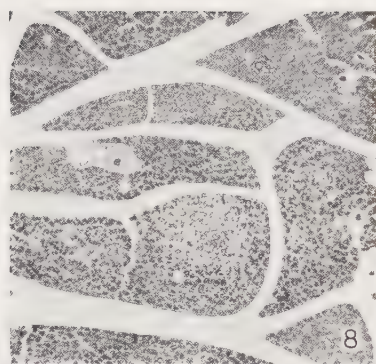
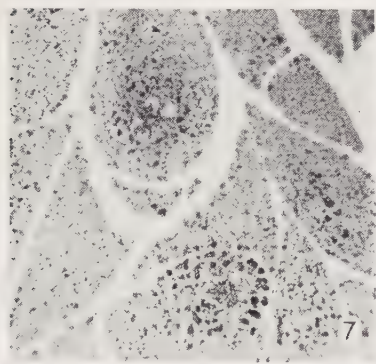
7 Effect of choline administration. The acinous cells, excepting those forming 'halos' around the islands, are all practically empty of granules.  $\times 75$ .

8 Effect of splanchnic stimulation, with the suprarenal glands intact. Note that the acinous cells in the immediate vicinity of the islands contain practically no zymogen granules. The islands stand out clearly.  $\times 75$ .

9 Effect of splanchnic stimulation, with the suprarenal glands intact. The granules of the  $\beta$ -cell cords stand out distinctly. In many places the boundary between the  $\beta$  cell cords and the surrounding acinous tissue is quite indistinguishable.  $\times 300$ .

10 Effect of splanchnic stimulation, with the suprarenal glands removed. The cells at the center of the lobules contain few zymogen granules; those at the periphery still retain some granules. There are almost no islands apparent in this group of lobules.  $\times 75$ .

11 Effect of adrenalin administration. The acinous cells are filled with granules. The islands stand out prominently.  $\times 75$ .







# THE EFFECTS OF INTRA-PERITONEAL INJECTIONS OF PITUITARY SUBSTANCES ON THE RATE OF TAIL REGENERATION IN FROG TADPOLES

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ONE PLATE (NINE FIGURES)

## INTRODUCTION

It has been shown by several investigators that the anterior lobe of the pituitary gland secretes a growth-promoting autocoid which tends to stimulate growth in amphibian larvae. Smith and Smith ('23) obtained a definite growth stimulation in frog tadpoles by intraperitoneal injections of saline extracts of the anterior lobe of the ox pituitary; Burns ('30) obtained an increase in the length of *Amblystoma* larvae by making transplants of the adult anterior lobe and Herrell ('34) found a decided increase in length in tadpoles of *Rana clamitans* by the use of an anterior pituitary extract.

A single paper has been found dealing with the effects of pituitary substances on regeneration processes in amphibian larvae. Herrell ('34) reported experiments showing the effects of an anterior pituitary extract ('Antuitrin G,' Parke-Davis) on the growth and regeneration of tail tissue in tadpoles of *Rana clamitans*. He placed tadpoles in water containing very small amounts of this extract, but gives no data as to the amount of the extract used. He found that the effect of pituitary treatment was dependent on the stage of regeneration at which the treatment was started. If treatment was begun before, or, at the time of tail section, the process of regeneration was retarded. If, on the other hand, treatment was begun several days after tail section and regeneration processes had commenced, then the extract offered a definite stimulus to the speed of regeneration.

Since these earlier experiments were reported more highly purified pituitary extracts have become available for experimentation. Too, it is the opinion of the writer that the intraperitoneal injection of these extracts is a more valid method of experimentation than an immersion of the animals in dilute solutions of these extracts. For these reasons the series of experiments to be described below were carried out to determine the effects of several pituitary extracts on the process of tail regeneration in frog tadpoles.

#### MATERIALS AND METHODS

Tadpoles of the common bullfrog, *Rana catesbiana*, were used in all the experiments. These tadpoles were collected from a single pool in the vicinity of Princeton, New Jersey in March and were judged to be about 10 months of age. Tadpoles selected for the experiments averaged 75 mm. in length and showed no signs of metamorphosis. In all the regeneration experiments approximately 25 mm. of the tail was removed. Amputations were made with the animals under chlorotone anesthesia. Experimental and control tadpoles were kept in large battery jars of water and under uniform conditions as regards temperature, light, background and feeding.

Two pituitary preparations were used in the experiments: 1) Parke-Davis Co. whole gland sheep pituitary extract, Research no. 096233-A.<sup>1</sup> This preparation supposedly contained all the pituitary secretions. 2) Anterior pituitary extract Squibb.<sup>1</sup> This latter extract is a commercial product which is very rich in the growth factor of the anterior pituitary, but also contains small amounts of the thyrotropic and sex complementary factors of this gland.

Animals under experimentation were injected intraperitoneally each day over the course of the experiments to be described. Control animals received an abdominal puncture with a hypodermic needle but no injections were made.

<sup>1</sup>The author wishes to express his thanks to the research departments of Parke-Davis Co. and of E. R. Squibb and Sons for the pituitary preparations used in this series of experiments.

Illustrations are unretouched photographs of animals preserved in formalin.

*The effects of whole gland sheep pituitary on tail regeneration in the frog tadpole*

The following experiment was devised to test the effects of daily injections of whole gland sheep pituitary extract on the rate of tail regeneration in frog tadpoles.

*Series A* (figs. 1, 2 and 3). Twenty tadpoles of *Rana catesbiana*, averaging 75 mm. in length and showing no signs of metamorphosis were selected for this experiment. These animals were, in all respects, similar to the animal shown in figure 1. Sections of the tail measuring 25 mm. in length were removed from each of these animals. Ten of these animals were then injected intraperitoneally with 0.1 cc. daily of whole gland pituitary extract for a period of 21 days. The remaining were uninjected controls.

The first deviation from the control animals shown by animals receiving pituitary injections was an intense blackening of the animals. It was earlier shown by Atwell ('19), Swingle ('21) and others that the pars intermedia of the pituitary gland produced a melanophore-expanding hormone which brought about a marked darkening of the animal. With the use of whole gland extracts, this reaction was to be expected in this series of experiments. This increase in pigmentation was noticeable in experimental animals 2 to 3 hours after injection and, if daily injections were stopped, the animals regained their normal pigmentation after a period of approximately 24 hours.

After 10 to 12 days of pituitary injections, experimental animals began to show definite metamorphic changes. The first and most noticeable change was the appearance and rapid growth of the hind limbs. Figure 2 shows an experimental animal after twenty-one daily injections of whole gland sheep pituitary extract. This animal possesses well-developed hind limbs, the mouth has lost its tadpole characteristics and the general body form has become more frog-like.

This animal should be compared with its control shown in figure 3.

Daily injections of whole gland pituitary extract produced a definite growth in these animals during the course of the experiment. Figure 2 shows a tadpole after 21 days of pituitary treatment and it will be noted that this animal is considerably longer than its control shown in figure 3. The head of this animal is very broad, the general contour of the body very irregular with a marked overgrowth of the dorsal tail fin. This growth increase does not follow the normal growth pattern but the animal appears 'overgrown' and very awkward in its movements.

Associated with the changes just described, daily injections of whole gland pituitary extract begun at the time of tail section causes a marked retardation in the rate of tail regeneration. Figure 2 shows a pituitary treated animal after twenty-one daily injections of pituitary extract. The amount of tail tissue regenerated amounted to 5 mm. as compared to 11 mm. regenerated by the control shown in figure 3. This is approximately the maximum amount of tissue regenerated by pituitary treated animals, as by this time metamorphosis has proceeded to a stage where a resorption of the tail sets in.

*Series B* (figs. 4 and 5). In a second series of experiments tail section was made on a group of tadpoles similar to those used in the preceding experiment. These animals were then allowed to regenerate tail tissue for 6 days at which time there was a noticeable amount of newly regenerated tissue present. At this juncture one-half of this group of tadpoles were started on daily injections of 0.1 cc. of whole gland sheep pituitary extract. The remaining one-half were uninjected controls. The rate of tail regeneration in these injected animals could not be distinguished from that of their controls. Figure 4 shows an animal of this series fixed 27 days after tail section and after twenty-one daily injections of whole gland pituitary extract. The amount of tissue regenerated by this animal was approximately 12 mm., the same amount as regenerated by its control shown in figure 5. As



a group, these injected animals did not differ from their controls, either in the rate of regeneration or in the total amount of tissue regenerated.

These experiments indicate that the effect of pituitary extract on the rate of tail regeneration in the frog tadpole is dependent on the stage of regeneration at which the injections are begun. If the administration of pituitary extract is started at the time of tail section, it retards the process of regeneration. On the other hand, if the regeneration process is allowed to start up and 6 days later pituitary treatment is begun, then regeneration proceeds normally. The growth of the animal as a whole is stimulated, but there is no discernible increase in the rate of tail regeneration. The probable significance of these results will be discussed in a later paragraph.

*The effects of anterior pituitary extract on tail regeneration  
in the frog tadpole*

The following experiment which was, in all respects, similar to the experiments made with whole gland pituitary extract was devised to test the effects of a highly purified anterior pituitary extract on tail regeneration in the frog tadpole. The extract used in this series of experiments was anterior pituitary extract (Squibb). As noted in an earlier paragraph, this extract is very rich in the growth factor of the anterior pituitary, but also contains small amounts of the thyrotropic and sex complementary factors of this gland.

*Series A* (figs. 6 and 7). Twenty tadpoles of *Rana catesbiana* were used in this experiment; 25 mm. of tissue was removed from the tails of each of these animals. Beginning with the date of operation, ten of these animals were given daily injections of 0.1 cc. of anterior pituitary extract for a period of 21 days. The remaining ten were uninjected controls.

Figure 6 shows a tadpole of this series after twenty-one daily injections of anterior pituitary extract. The uninjected control of this animal is shown in figure 7. One of the most

noticeable deviations from the control is the increased pigmentation shown by the injected animals. They tend to become as dark as the animals injected with whole gland extract. Herrell ('34) reported that tadpoles of *Rana clamitans* immersed in dilute solutions of anterior lobe extract became very light in color, becoming almost depigmented after a period of treatment. The intraperitoneal injection of anterior lobe extract in this series of experiments produced just the opposite effect, a marked increase in pigmentation. Although it is a well-established fact that the melanophore-expanding hormone is a product of the pars intermedia of the pituitary gland, it is able, however, to make its way into the anterior lobe of the gland as Smith ('25), Blacher ('27) and others have reported pigmentary changes in various amphibian larvae after injections of anterior lobe substances.

Reference to figure 6 also shows that anterior lobe extract brings on a precocious metamorphosis in injected animals. This is most noticeable in the appearance and rapid growth of the hind limbs. This behavior was to be expected as the thyrotropic factor of the anterior pituitary is known to stimulate various amphibian larvae to a precocious metamorphosis.

Repeated injections of anterior lobe extract bring about marked changes in the size and general body form of the larvae. The increase in length is accompanied by striking changes in the general shape of the body. This is well illustrated by the animal shown in figure 6 which should be compared with its control shown in figure 7. The body of the animal becomes very rough, the head broad and, most striking of all, the tail fin becomes enormously expanded. This overgrowth of the tail fin is much more pronounced than in the animals injected with whole gland pituitary extract.

Associated with this marked overgrowth of the tail, anterior pituitary extract causes a marked retardation in the rate of tail regeneration in these animals. The injected animal shown in figure 6 has regenerated only 5 mm. of tail tissue, while its uninjected control shown in figure 7 has regenerated

approximately 12 mm. of tissue. However, it should be noted that the tail fin of the injected animal is much broader than in the control animal so that the difference in the volume of tissue regenerated is not as great as the figures might indicate.

*Series B* (figs. 8 and 9). In a second series of experiments 25 mm. of tissue was removed from the tails of each of twenty tadpoles. Regeneration was allowed to proceed normally for 6 days. At the end of this period daily injections of 0.1 cc. each of anterior pituitary extract were begun on ten of the larvae and continued for a period of 21 days. The remaining ten were used as controls. Figure 8 shows an animal of this series fixed 27 days after tail section and after twenty-one daily injections of anterior pituitary extract. This animal, while showing very definite metamorphic changes, has regenerated an amount of tissue equal to that of its control shown in figure 9. The length of the regenerate in each case is approximately 14 mm. The injected animal shown in figure 8 shows a marked reduction in the size of the tail fin due to the rapid onset of metamorphosis. Here again, it seems that in cases where tail regeneration is allowed to start up before pituitary treatment is begun, pituitary substances have little or no effect on the rate or amount of tail tissue regenerated.

#### DISCUSSION

The process of tail regeneration in the frog tadpole can be divided into three phases: 1) a period of reorganization immediately after the amputation of tail tissue, during which there is a reorganization of the formed structures at the cut surface of the tail preparatory to the proliferation of fresh tissue; 2) a period of proliferation during which there is a proliferation of fresh tissue from the cut surface of the tail to form the regeneration blastema, and 3) a final period in which this newly proliferated tissue of the blastema becomes differentiated into the formed structures of the tail.

The experiments described above indicate that the effect of pituitary extracts on tail regeneration is dependent on the stage of regeneration at which the injections of these substances are begun. If the injections are begun at the time of tail section, a hyper-pituitary condition appears to retard regenerative processes. On the other hand, if the period of reorganization is allowed to go to completion and an active proliferation of fresh tissue has begun at the time injections are started, then regeneration proceeds in a normal manner. It appears that excess pituitary substances, in some way retard this initial process of reorganization of the formed structures at the cut surface of the tail.

It is also interesting to note that in the case of pituitary-treated animals where regeneration is allowed to start up before pituitary treatment is begun, the tail regenerates at approximately the same rate as in the control animals. On the other hand, pituitary treatment causes a marked increase in the growth of the body as a whole, injected animals becoming very long with a marked increase in the height of the tail fin. Thus, while the formed structures of the body are stimulated to a much more rapid growth rate by pituitary treatment, regenerating tissues do not show any such acceleration. It thus appears that this so-called growth hormone of the anterior pituitary gland acts in an entirely different manner on formed structures of the body as compared with embryonic-like regenerating tissues. It appears to stimulate growth in formed structures of the body, but exerts little or no influence on the growth of undifferentiated regenerating tissues.

#### CONCLUSIONS

1. Intraperitoneal injections of pituitary extracts into tadpoles of *Rana catesbiana* bring about marked growth changes in the animal. There is a definite growth stimulation, but this increase in growth does not follow the normal growth pattern. There is a tendency toward an overgrowth of structures, especially of the tail fins.



2. Injections of either whole gland sheep pituitary extract, or of a purified anterior pituitary extract cause an expansion of the melanophores of the animal, resulting in an intense blackening of the animal. If injections are stopped, the animals regain their normal pigmentation after a period of approximately 24 hours.

3. The effect of pituitary extracts on the regeneration of the tail is dependent on the stage of regeneration at which the injections are started. If injections are started at the time of tail section there is a marked retardation in the rate of regeneration. If, on the other hand, the tail is allowed to regenerate for a period of 6 days and then pituitary treatment is started, there is no apparent effect, either on the rate or amount of tissue regenerated.

4. Evidence points to the fact that the so-called growth hormone of the pituitary gland has a different effect on formed structures of the body than that on embryonic-like regenerating tissues. It appears to stimulate the growth of formed body structures, but does not accelerate the rate of regeneration of new structures.

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## PLATE 1

### EXPLANATION OF FIGURES

The level of amputation in each case is indicated by line A--B.

- 1 *Rana catesbiana* tadpole at the time of tail amputation.
- 2 Injected animal after twenty-one daily injections of whole gland sheep pituitary extract. Injections begun at the time of tail amputation.
- 3 Uninjected control of the animal as shown in figure 2.
- 4 Injected animal after twenty-one daily injections of whole gland sheep pituitary extract. Injections begun 6 days after tail amputation.
- 5 Uninjected control of the animal shown in figure 4.
- 6 Injected animal after twenty-one daily injections of anterior pituitary extract. Injections begun at the time of tail amputation.
- 7 Uninjected control of the animal shown in figure 6.
- 8 Injected animal after twenty-one daily injections of anterior pituitary extract. Injections begun 6 days after tail amputation.
- 9 Uninjected control of the animal shown in figure 8.





# HISTOLOGY OF THE OVIDUCT OF THE FOWL IN RELATION TO VARIATIONS IN THE CONDITION OF THE FIRM EGG ALBUMEN

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TWO PLATES (EIGHT FIGURES)

## INTRODUCTION

The microscopic structure of the oviduct of the fowl has received relatively very little attention or consideration in the past. Surface ('12) published an excellent paper dealing with the histology of the avian oviduct. Bradley ('28) extended somewhat the work of Surface, and Richardson ('35) has published a detailed account of the glands of the oviduct, especially those of the uterus.

In recent years other investigations have dealt with the appearance of variations in the edible portion of the fresh egg and various means of measuring and classifying them. Studies by Asmundson ('31), Asmundson and Jervis ('33) and Asmundson and Burmester ('36) on the physiology of the oviduct were made to ascertain, by surgical removal of various portions, which sections of the organ secreted the various types of albumen. These are the chalaziferous layer, inner thin, firm and outer thin albumen. McNally ('33, '34) and Scott, Hughes and Warren ('37) studied the chemical composition of the egg at various stages of its development in relation to the known physiology of the oviduct.

There is general agreement among the workers investigating the quality of eggs (literature reviewed by Van Wagenen and Hall, '36), that consistent individual differences in the condition and quantity of the firm-albumen are inherited,

being apparently affected by multiple genes. These differences are extremely consistent for any individual bird and are not influenced by any environmental factors except for a slight seasonal variation. However, those interested in the quality of the egg have paid very little attention to the oviduct in which it is formed. It therefore seemed fitting to examine those processes which precede the laying of the egg and which must make important contributions to the variations in the quality of fresh eggs.

Previous investigations include the histology of the oviduct, the effects of resection at various levels upon the constituents of the succeeding eggs, and the chemical variations in the thick and thin albumen and between the albumen of immature and mature eggs. These all suggest that the albumen-secreting region, especially its mucin-secreting cells, plays an important role in the production of firm albumen and therefore the present investigation is centered upon this region of the oviduct.

The writer acknowledges the many helpful suggestions and criticisms from Prof. F. B. Hutt under whose direction this study was conducted.

#### MATERIALS AND METHODS

Single comb white Leghorns which were finishing their first year of laying were used in this study. Studies were carried out on the eggs from eighty-nine females, records being made of the weights, volumes of the various layers (by pipette method), the height of the unbroken firm-albumen envelope (by means of a tripod micrometer) and of the score for the condition of the firm albumen according to the scale suggested by Sharp ('32) and Van Wagenen and Wilgus ('35). These authors are of the opinion that the score is the best method of classifying the 'interior egg quality' even though it is not subject to exact measurement, being influenced to a certain degree by the individual doing the scoring. Fourteen birds were chosen from the eighty-nine which could be paired



with reference to similar weight of egg and at the same time which were at extremes as to the condition of the firm albumen (figs. 3 and 4). The classification of the firm egg albumen for any bird is based upon the averages of three or more eggs (with one exception). For each of the birds chosen for the histological study the eggs showed remarkable uniformity of all internal characteristics. These birds were taken for study within 1 hour after laying, and, with the exception of one pair, at a time when there was the normal expectancy that they would have laid the following day. This was based upon the hour of previous laying and cycle of production, both determined by trapnest records. The entire oviduct from each bird was removed, relieved of the surrounding connective tissue and ligaments, and then split along the line of the attachment of the superior ligament. The oviduct was spread out and pinned down in a paraffin-coated glass plate to prevent excessive shrinking or curling and then fixed for approximately 5 hours in 10% formalin. Blocks of tissue, 1 cm. square, were cut from the side of the oviduct at fifteen different evenly spaced levels and transferred to Gilson's fluid for 15 hours. Following washing in running water, dehydration in alcohol, clearing in xylol and embedding in paraffin, the sections were cut to a thickness of  $8\mu$  and stained with Ehrlich's hematoxylin followed by muci-carmin.

Preliminary studies had shown differences in the intensity of staining of the goblet cells in the albumen-secreting region. To eliminate any differences possibly resulting from technique throughout this study sections from the corresponding blocks of tissue of each pair of birds were stained (back to back) so that any observable differences in staining would be due to the tissue itself and not to the technique.

The last egg laid by each of ten of the fourteen birds was hard boiled, while in a vertical position, for 5 minutes. Sections 1 cm. square were cut from the air-cell surface through all the layers of the coagulated albumen and including the vitelline membrane. These were fixed, dehydrated, cleared and embedded as described above. The egg-albumen sections were stained with muci-carmin or thionine blue.

In preliminary studies various stains were used of which Ehrlich's hematoxylin followed by mucicarmine gave the best differential stain. The goblet cells in the infundibulum and the albumen-secreting region (figs. 5, 6 and 8) were stained red by the mucicarmine stain whereas no other cells in the oviduct, not even the goblet cells of the isthmus and uterus (fig. 7), were so stained. The nuclei in all the cells were stained by the hematoxylin.

#### OBSERVATIONS

##### *A. Egg albumen*

The sections through the layers of the coagulated egg albumen show rather clearly the structural bases for the observed variations in the condition of the firm albumen of the fresh eggs. The firm albumen of good consistency (fig. 1) consists of numerous closely packed mucin-like fibers, characterized by their ability to take the specific mucin stains. The firm albumen of poor consistency (fig. 2) consists of relatively few sparsely distributed mucin fibers. A study of twenty-eight additional eggs shows that between these extremes a gradation in the consistency of albumen is paralleled by a gradation in the number and distribution of the mucin fibers. The microscopic picture is apparently a true indication of the consistency of the firm albumen. Dried specimens of uncoagulated firm albumen, when stained with mucicarmine, show the presence of these fibers with their typical staining reactions.

##### *B. Oviduct*

The stained sections from the oviduct show that the goblet cells on the surface of the albumen-secreting region contain a material which is similar in nature, as indicated by its reaction to the mucin stains, to the mucin fibers which form the differentiating bases for the observable variations in the fresh firm albumen. These cells containing mucin are rather low and in the anterior portion of the albumen-secreting region alternate with hair cells. Proceeding caudad from

about the middle of the albumen-secreting region the height of these goblet cells increases greatly with a maximum being reached at the junction of the albumen-secreting region with the isthmus. That the posterior half of the albumen-secreting region contributes most of the formation of the firm albumen is evidenced by the physiological studies of Asmundson and Burmester ('36) in which they demonstrated that the removal of a section from this region resulted in a marked reduction in the amount of firm albumen secreted. The greater the size of the section removed the greater was the reduction in the amount of firm albumen secreted. They suggested that there may be some relationship between their findings and the height of the goblet cells in this region, as reported by Bradley ('28). Toward the caudal portion of this region the hair cells become relatively smaller and in many places are not readily discernible. The enlarged goblet cells containing mucin appear to form a continuous band on the surface of the longitudinal folds of the oviduct. The presence of hair cells is indicated by the second row of nuclei which can be seen at about the middle of the epithelial layer in figures 6 to 8.

That these goblet cells are the source of the material that forms the distinguishing mucin fibers is proven by sections which show the actual formation of a mucin fiber (fig. 8). The material within the goblet cells lining the small crypt near the junction of the albumen-secreting region with the isthmus can be seen in the process of being drawn out from the cells to form a large fiber which takes the mucin stain. The fate of the material secreted from these unicellular glands has, in the past, not been definitely known. Bradley ('28) stated that the material secreted in the form of large droplets disintegrated immediately into fine granules. Richardson ('35) stated that it appeared as strings in close association with the lining epithelium. Surface ('12) described the process, "the secretion from the goblet cells can often be seen pouring out in little streams from each cell."

A study of these important goblet cells showed an apparent difference in their heights in birds laying eggs with firm albumen of a good consistency as compared to those laying eggs with firm albumen of a poor consistency (figs. 5 and 6). In order to determine if this difference actually existed, and, if so, to secure a means of presenting the data, measurements with a calibrated ocular micrometer were made of the height of these cells. Twenty measurements of cells which had been cut at right angles to their base were taken from each of the eight sections from the albumen-secreting region of the fourteen oviducts. As the heights of the goblet cells vary somewhat, depending upon their position on the longitudinal folds,

TABLE 1

*Mean heights of goblet cells at different levels of the oviduct. Each of the sixteen means represents 140 measurements*

| <i>Level of oviduct<br/>(proceeding caudad<br/>from 11 to 100)</i> | <i>Height of cells in<br/>oviducts producing</i> |                                     | <i>Difference<br/>good—poor<br/>microns</i> |
|--------------------------------------------------------------------|--------------------------------------------------|-------------------------------------|---------------------------------------------|
|                                                                    | <i>Good<br/>albumen<br/>microns</i>              | <i>Poor<br/>albumen<br/>microns</i> |                                             |
| 11                                                                 | 11.6                                             | 11.2                                | 0.4                                         |
| 25                                                                 | 12.1                                             | 11.7                                | 0.4                                         |
| 37                                                                 | 13.0                                             | 10.9                                | 2.1                                         |
| 52                                                                 | 17.1                                             | 14.3                                | 2.8                                         |
| 65                                                                 | 24.6                                             | 21.8                                | 2.8                                         |
| 78                                                                 | 26.9                                             | 24.5                                | 2.4                                         |
| 91                                                                 | 30.1                                             | 29.2                                | 0.9                                         |
| 100                                                                | 34.8                                             | 33.1                                | 1.7                                         |

the twenty measurements were distributed over the entire section and should therefore represent a fair average. In any one bird the height of the goblet cells varied but little in the anterior third of the albumen-secreting part of the oviduct. The average height of 840 cells from all birds was  $11.8\mu$  in this region. Halfway down the oviduct, the height of 280 cells from fourteen birds was  $15.7\mu$ . At the extreme caudal end of the albumen-secreting portion the average height of 280 cells was  $33.9\mu$ . The bird producing the eggs with a good condition of the firm albumen possesses a definitely higher goblet cell throughout most or all of the albumen-secreting region. This difference was consistent for each of the seven pairs studied.



The average heights of 140 goblet cells at each of eight levels from seven oviducts in each series, one producing good albumen, the other poor, are shown in table 1. The various levels are expressed in per cent and measured as (distance from anterior end of the albumen-secreting region to the level of the section  $\times 100$ ) divided by length of the albumen-secreting portion.

Application of Student's test for significance of differences between paired observations (Fisher, '34) yields a value for  $P$  of  $<.01$ , indicating a highly significant difference in height between the goblet cells of the two series. It was also noted that the greater the difference in the consistency of the firm albumen between the birds of any pair (as based upon the score) the greater was the difference in the height of these mucin-secreting cells.

#### DISCUSSION

The use of the height of a cell as an indication of its size and probable volume of secretion is of course open to criticism. But, in view of the facts here presented, there can be no doubt that there is a consistent relationship between the height of the mucin-containing goblet cells of the albumen-secreting region and the condition of the firm albumen produced. The latter is dependent upon the number and distribution of the mucin fibers present. The accumulation of the mucin material within the goblet cells must reach its maximum just prior to its secretion. That the observed variation in the size of the cells is not just a manifestation of the varying stages of accumulation of the prosecretion seems probable in view of the fact that the birds were taken immediately after laying at which time the oviduct is reaching its maximum storing capacity, as the firm albumen for the succeeding egg would be laid down within approximately 4 hours (Warren and Scott, '34).

Observations recorded in this study provide a better understanding of the actual formation of the various layers of the albumen. The comparatively low mucin-containing goblet cells in the anterior half of the albumen-secreting region produce mucin fibers, the strands of mucin-like protein reported



by Almquist and Lorenz ('32) which, with the counterclockwise rotation of the egg as it traverses the oviduct (Almquist, '36), are twisted together to form the chalazae. What is left out of the chalazae becomes the inner thin layer of albumen. The posterior part of the albumen-secreting region with its high goblet cells produces the firm albumen. The junction between the firm and the outer thin albumen is often rather sharp with a closely packed band of long mucin-like fibers forming the outer zone of the firm layer. The outer thin albumen, which is produced in the uterus and does not contain mucin when secreted, has been seen to contain occasionally a few scattered fibers which have apparently been separated from the firm albumen. This would account for the small amount of mucin found in the outer thin albumen by McNally ('33).

#### CONCLUSIONS

1. The number and distribution of the mucin-like fibers form the structural bases for the observed variations seen in the condition of the firm-albumen layer of fresh eggs.

2. These mucin fibers are produced by the goblet cells lining the albumen-secreting region of the oviduct, particularly by those in the posterior half of that organ.

3. The height of these cells averages  $11.4\ \mu$  in the anterior portion of the albumen-secreting region,  $15.7\ \mu$  in the middle region,  $30\ \mu$  in the posterior region and reaches a maximum of  $33.9\ \mu$  at the junction with the isthmus.

4. Fowls producing eggs with firm albumen of good condition possess a consistently higher goblet cell throughout the albumen-secreting region than those producing a more watery type of firm albumen.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Section of heat coagulated firm albumen of good consistency from a fresh egg with a score of 1.50, stained with thionine blue. Note large number of closely packed mucin fibers.  $\times 30$ .

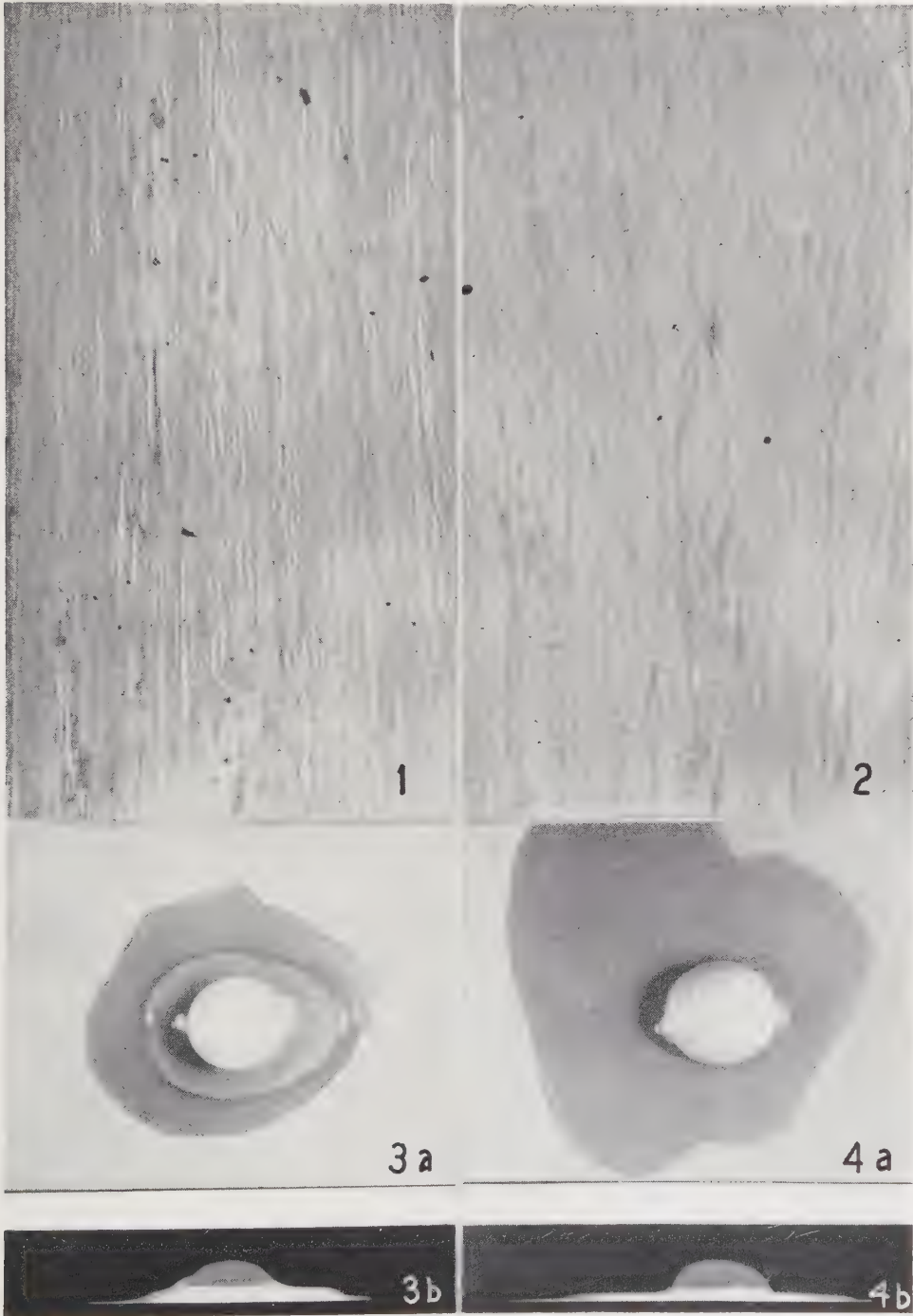
2 Section of heat coagulated firm albumen of poor consistency from a fresh egg with a score of 4.00, stained with thionine blue. Note, in comparison to figure 1, the scarcity and wide separation of the mucin fibers.  $\times 30$ .

3<sup>1</sup> a) Surface view of a fresh egg with firm albumen of good consistency, score of 1.50. The firm albumen envelope retains its shape and does not break away from the yolk. b) Silhouette view of egg in figure 3 a. The firm albumen stands up well around the yolk.

4<sup>1</sup> a) Surface view of a fresh egg with firm albumen of poor consistency, score of 4.00. The firm albumen envelope is not able to maintain its shape. The firm albumen breaks away from the yolk and covers a large area. b) Silhouette view of egg in figure 4. The firm albumen can not maintain its normal position in relation to the yolk because of its lack of sufficient internal structure.

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<sup>1</sup> Figures 3 and 4 reproduced with permission of A. Van Wagenen and H. S. Wilgus, Jr.



## PLATE 2

### EXPLANATION OF FIGURES

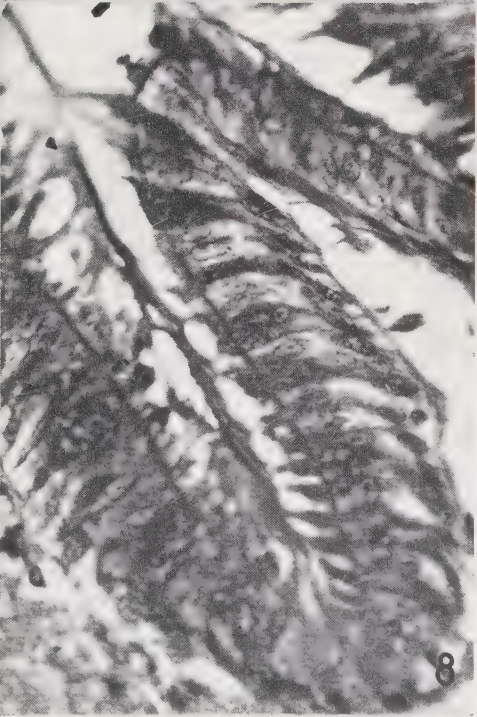
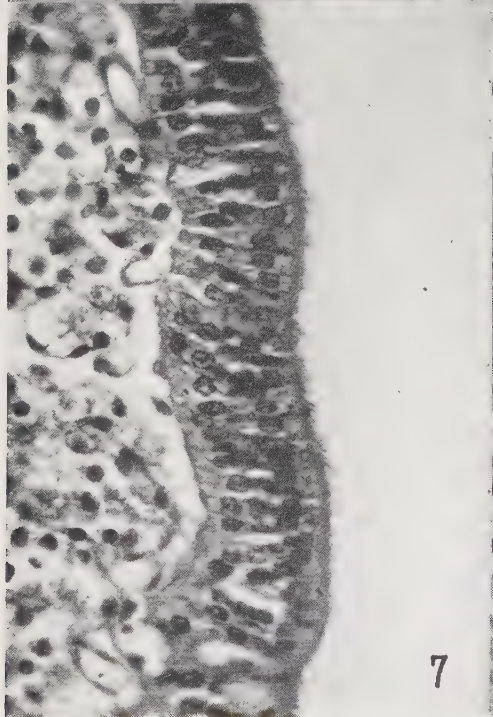
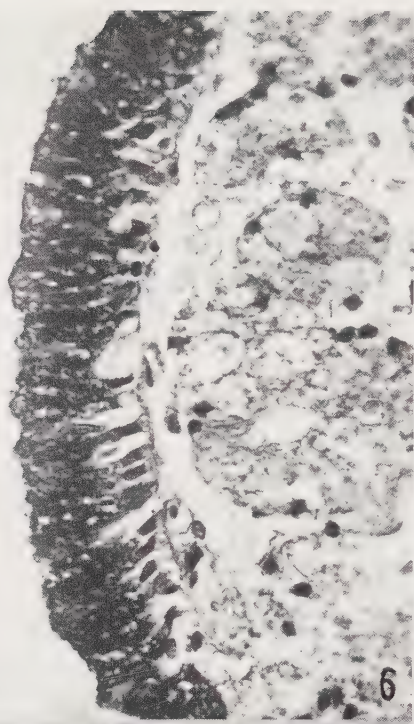
5 Section of the posterior albumen-secreting region at a level of 91, from a bird laying eggs with firm albumen of good consistency, stained with Ehrlich's hematoxylin and muci-carmin. Note the large goblet cells in the high epithelium containing a dark-staining mucin-like material.  $\times 440$ .

6 Section of the posterior albumen-secreting region at a level of 91, from a bird (same size egg as for the bird shown in figure 5) laying eggs with firm albumen of poor consistency, stained with Ehrlich's hematoxylin and muci-carmin. Note the much smaller goblet cells with their dark-staining mucin-like material in the lower epithelium, when compared to figure 5.  $\times 440$ .

7 Section of uterus, stained with Ehrlich's hematoxylin and muci-carmin. Note the double row of nuclei in the epithelium and the lack of dark-staining mucin-like material.  $\times 440$ .

8 Section from extreme caudal portion of the albumen-secreting region, stained with Ehrlich's hematoxylin and muci-carmin. The dark-staining mucin-like material in the goblet cells of the crypt is in direct continuity with the mucin fiber of the lumen. The material secreted by the goblet cells forms the mucin fibers that are so characteristic of the firm albumen.  $\times 440$ .







# ATROPHY OF THE ADRENAL CORTEX IN THE RAT PRODUCED BY ADMINISTRATION OF LARGE AMOUNTS OF CORTIN

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FOUR FIGURES

Morphologic changes in certain of the ductless glands have been induced by administration of large amounts of the hormone secreted by the gland. It was shown by Moore and Price ('32) that injection of rats with large amounts of either the follicular hormone or the testicular hormone induced atrophy of the gonads of either sex. Loeb, Bassett and Friedman ('30) observed regressive changes in the thyroid gland following administration of excessive amounts of thyroid substance.

This report covers the results of experiments wherein we have studied the effect of administration of large amounts of the hormone of the adrenal cortex and, also, of two crystalline compounds separated from the adrenal gland, which possess cortin-like activity, on the size and morphology of the adrenal glands in white rats.

## METHODS

Male rats of the Wistar strain which weighed 180 to 182 gm. were used. They were kept in separate cages and maintained on an adequate commercial diet which contains 0.24% of sodium and 1.0% of potassium. Cortin was administered in

the drinking water. Our selection of this method of giving cortin was based on our observation (Ingle and Higgins, in press) that regeneration of an enucleated adrenal gland was inhibited more effectively when cortin was given orally than when given three times daily by subcutaneous injection. The mode of administration is a matter of convenience. If injections had been given continuously at short intervals, cortin probably would have been as effective as when given by mouth.

In the first experiment, forty rats were provided with cortin for a period of 7 days; the rats were divided into groups of five on the basis of the amount of cortin received each day. The amounts given daily to each rat of its respective group were 1, 2, 3, 4, 5, 6, 7 and 10 cc. Each rat of an additional group of six received daily 10 cc. of cortin orally and 1 cc. of an anterior pituitary extract of the adrenotropic hormone<sup>1</sup> by intraperitoneal injection. Each cubic centimeter of cortin contained the extract prepared from 75 gm. of whole adrenal gland.

In the second experiment, each rat of a group of eighteen was given daily 10 cc. of the same preparation of cortin. Two animals were killed at each of the following intervals: 1, 2, 3, 4, 5, 6, 7, 14 and 28 days.

In experiment 3, two chemically pure crystalline compounds known in this laboratory as 'A' and 'B',<sup>2</sup> which have been isolated from the adrenal cortex and which have been shown to possess cortin-like activity, were tested for their effect on the adrenal gland of the normal rat. Two rats were given 3.3 mg. of compound A daily for 7 days and two rats were given 3.3 mg. of compound B daily for 7 days. These compounds were added directly to the drinking water.

All animals were killed by exsanguination. Both adrenal glands were removed carefully, weighed to the nearest milligram, and fixed and stained for histologic study. The pituitary gland, thyroid gland, thymus gland, gonads, seminal

<sup>1</sup> The hormone used was supplied through the courtesy of Oliver Kamm, Detroit, Michigan.

<sup>2</sup> Reichstein also has separated compound B and has named the compound 'cortico-sterone.' Compound A is the same as B except for a ketone group on C<sub>11</sub> in place of a hydroxyl group.

vesicles, kidneys, liver and spleen also were removed and weighed. It is evident that removal of small glandular organs, such as the adrenals, and freeing them of all adherent adipose and connective tissue prior to weighing, necessitates loss of moisture and, thus, involves some error; therefore, the values given for weight of the adrenal glands should not be considered as absolute. These weights have been obtained by procedures which we have standardized and the margin of

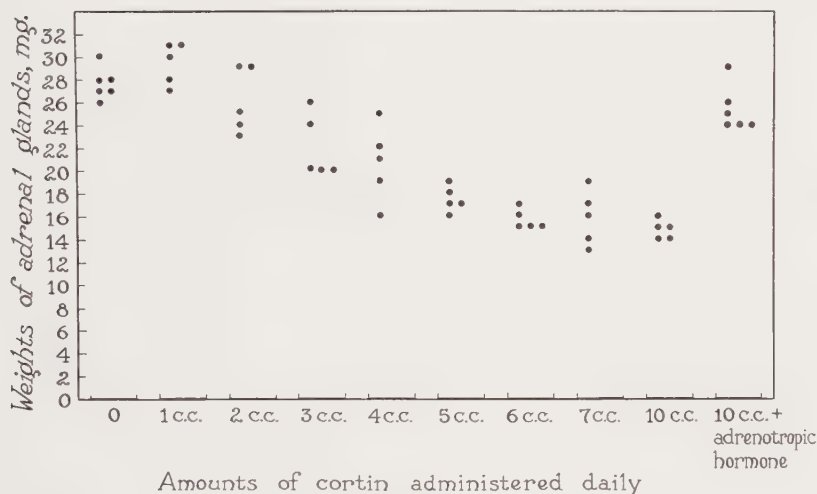


Fig. 1 Combined weights of adrenal glands of normal male rats which had received cortin daily for 7 days.

error in the weight of the two adrenal glands we believe does not exceed 2 mg.

## RESULTS

The data derived from experiment 1 are graphically shown in figure 1. There was a marked relationship between the amounts of cortin given and the weights of the two adrenal glands. In general, the larger the amounts of cortin consumed, the smaller the weights of the adrenal glands at the end of the period of 7 days. The average combined weight of the two adrenal glands of six normal non-cortin



treated rats was 27.7 mg. At the end of 1 week, the five rats consuming 2 cc. of cortin daily had adrenal glands which weighed an average of 26.0 mg. The five rats which consumed 5 cc. of cortin daily had adrenal glands which, after the period of 7 days, weighed on the average 17.4 mg. although those which had received 10 cc. daily had adrenal glands which averaged 15.0 mg.

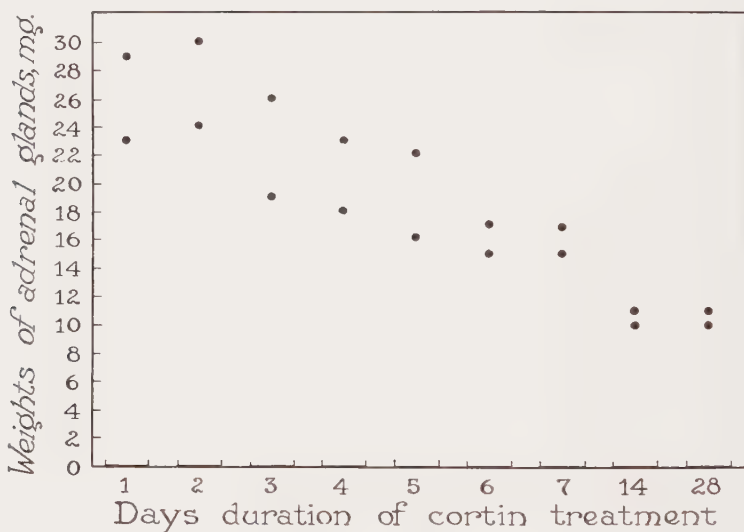


Fig. 2 Combined weights of adrenal glands of normal male rats which had received 10 cc. of cortin daily.

Examination of the six animals which received the adreno-tropic fraction in addition to the 10 cc. of cortin daily, gave no evidence that the adrenal glands were atrophic. The average of the combined weights of the adrenal glands, of these six rats after the 7 days was 25.4 mg., a figure slightly less than that found in normal untreated rats (fig. 1).

The data derived from experiment 2 are graphically shown in figure 2. These data support those derived from the first experiment and show that, as the time of administration of cortin was increased, the weights of the adrenal glands were correspondingly less. This is true up to the fourteenth day.

The two animals carried for 14 days longer, during which period each received an additional 140 cc. of cortin, did not show any greater degree of atrophy of the adrenal glands than did those killed on the fourteenth day.

The results of the third experiment, in which the pure crystalline compounds A and B were administered orally, were

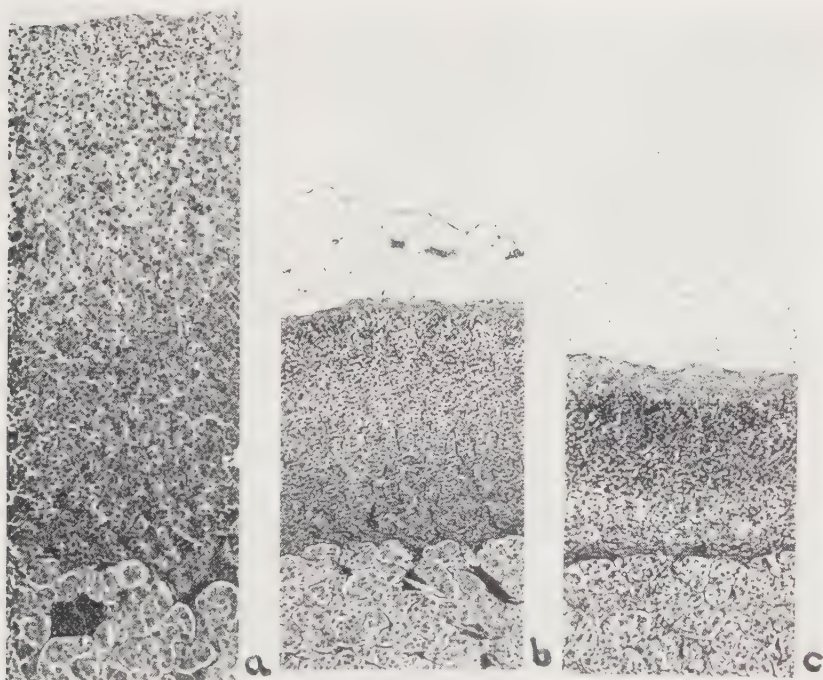


Fig. 3 Sections of cortices of adrenal glands ( $\times 85$ ); *a*, normal male rat; *b*, male rat, receiving massive doses of cortin; *c*, male rat, hypophysectomized, showing adrenal atrophy.

like those obtained when the total extract of the gland was given. A degree of atrophy of the adrenal gland, comparable to that seen in the other two experiments, was produced by daily administration of 3.3 mg. of either compound A or B for 1 week. The average of the combined weights of the two adrenal glands removed from the two rats given compound A for 7 days was 16 mg.; the average combined weight of the

glands in the two animals which received compound B was 15 mg.

Section of these glands showed clearly that atrophy indicated by the loss in weight of the adrenal glands occurred in the cortex. Comparison of a section of a normal gland with one taken from an animal which had received massive doses

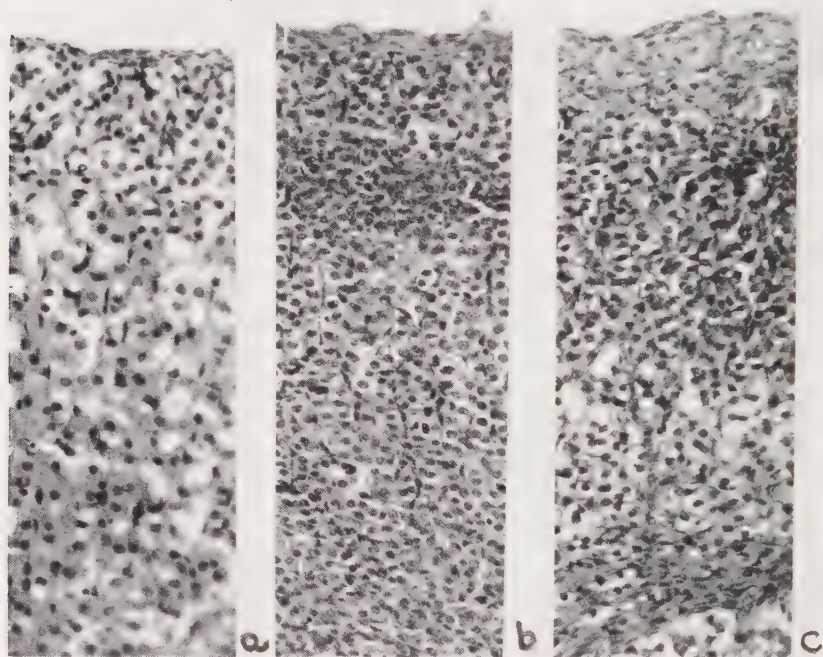


Fig. 4 Sections of peripheral cortices of adrenal glands ( $\times 235$ ); *a*, normal male rat; *b*, male rat, receiving massive doses of cortin; *c*, male rat hypophysectomized.

of cortin, indicated that the cortex of the latter was about half the depth of the former (fig. 3). The atrophy of the cortex of the cortin-treated animal was nearly as great as that which occurred in the adrenal gland of a completely hypophysectomized animal (fig. 3, *c*). Cells of cortices of animals which had received the maximal amounts of cortin were much smaller than cells of the normal gland (fig. 4). The nuclei

of cells were closely packed. Cytoplasmic bodies were very small and there were no lipoid inclusions such as occur in the normal cortex. Examination of sections gave evidence of great contraction of the cortex. The capsule of such glands was thickened and somewhat convoluted. Those animals which were treated with large amounts of cortin (5 to 10 cc. daily) showed an early loss in body weight in amounts up to 20 gm. This loss was recovered gradually but subsequent growth was retarded below the rate characterizing the normal untreated rat. No data on the intake of food was obtained in these experiments.

The thymus gland showed a marked progressive atrophy in those rats which were treated with large amounts of cortin (5 to 10 cc. daily). There was an increase in the weight of the liver above the normal values. This increase was most marked in the early stages of treatment. No change was noted in the gonads or seminal vesicles. The mean of our data on the weights of the pituitary body indicates that a small loss in the weight of this organ may have occurred during treatment with massive doses of cortin, but this was not clearly established.

#### DISCUSSION

Earlier studies (Howard and Grollman, '34; King, '37) on the effect of administration of comparatively large amounts of cortin to rats failed entirely to corroborate the results that have just been described. We suggest, however, that differences in the amounts of active material administered and in the methods of giving the cortin will explain adequately the differences which have been encountered.

The amounts of cortin which are required to produce atrophy of the adrenal cortex are very large when compared with the minimal amounts which will produce a physiologic response in the adrenalectomized animal. The cortical extract used in these experiments has been shown by the Ingle rat test to enhance the capacity for work of the adrenalectomized rat when injected subcutaneously in amounts of 0.2 cc.



twice daily. The crystalline compounds which we used also were shown to enhance the capacity for work of adrenalectomized rats, if injected subcutaneously in amounts of 0.15 mg. twice daily. But it must be recognized that the maintenance of life and a state of apparent good health in the adrenalectomized animal by replacement therapy are not valid criteria that all of those functions normally influenced by the adrenal gland have been restored.

Our own observations (Ingle and Higgins, in press) on inhibition of regeneration of the adrenal gland by administration of cortin and the studies of Long on the amounts of cortin required to induce glycosuria in the adrenalectomized depancreatized rat seem to demand the conclusion that there is a wide discrepancy between the minimal amounts of cortin required to maintain life and those amounts required to replace completely all functions of the intact adrenal glands. Recognition of this fact indicates a need for reinvestigation of the experimental situations in which apparent failure of the life-maintaining hormone of the adrenal cortex to maintain the physiologic normality of the adrenalectomized animal has led the investigator to postulate the presence of other hormones in the adrenal cortex.

Schacher, Browne and Selye ('37) have observed an inhibition of somatic growth and atrophy of the thymus gland following administration of estrogenic substances to the rat. The chemical nature of compounds A and B (Mason, Hoehn, McKenzie and Kendall, '37) is similar to that of the sex hormones and all of these compounds may have their effect on the thymus gland and on growth of the body by the same mechanism. This possibility is, of course, only speculative.

In a preliminary report we (Ingle and Kendall, '37) suggested that the adrenotropic activity of the pituitary gland is suppressed by an excess of cortin. We have shown that administration of an adrenotropic fraction of an extract of the anterior pituitary gland simultaneously with cortin, prevented the atrophy of the adrenal gland that accompanied treatment with cortin alone. The assumption that the anterior pituitary



gland is influenced, in its secretory activity, by either a deficiency or an excess of cortin and that it influences the activity of the adrenal cortex to meet the physiologic requirements of the organism for cortin, will explain the known facts regarding hyperplasia and atrophy of the adrenal gland. However, the assumption is no more than a working hypothesis.

#### SUMMARY AND CONCLUSIONS

The purpose of this study was to determine the effects, upon the adrenal glands of normal white rats, of massive doses of cortin which were administered for varying periods of time.

Large amounts of an active extract of the adrenal gland have been given to rats by mouth for a period of 1 to 28 days. The smallest amount was 1 cc. per day for 7 days and the largest amount was 10 cc. per day for 28 days. Likewise, two crystalline compounds which were separated from the adrenal cortex, known in the literature as compounds 'A' and 'B' were given orally in amounts of 3.3 mg. daily for 1 week. Adrenotropic hormone was given to one group of six animals during the time that each received 10 cc. of the cortin preparation daily. The following conclusions have been made:

1. Atrophy of the adrenal gland was induced in normal rats by administration of large amounts of cortin. Atrophy of the glands became more marked as the amounts of cortin were increased.

2. Administration of the crystalline compounds A or B produced, in 1 week, degrees of atrophy of the adrenal gland essentially similar to those attained when large amounts of cortin were given. This result definitely shows that atrophy of the adrenal gland is produced by the same crystalline compound which will maintain the adrenalectomized rat in a normal condition.

3. Atrophy of the gland was restricted to the cortex. The cells were much smaller than those of the normal gland. Cytoplasmic bodies were reduced in size and there were no lipid deposits in the cells. The extent of atrophy of the glands of animals treated with cortin resembled that which occurs in totally hypophysectomized animals.

4. When an adrenotropic fraction of the anterior pituitary gland was given simultaneously with the cortin, atrophy of the adrenal gland did not occur.

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## BOOK REVIEW

DIABETES INSIPIDUS AND THE NEURO-HORMONAL CONTROL OF WATER BALANCE. A CONTRIBUTION TO THE STRUCTURE AND FUNCTION OF THE HYPOTHALAMICO-HYPOPHYSEAL SYSTEM. By Charles Fisher, Ph.D., W. R. Ingram, Ph.D. and S. W. Ranson, Ph.D., M.D., Institute of Neurology, Northwestern University Medical School, Chicago. Edwards Brothers, Inc., Ann Arbor, Michigan, 1938. x + 212 pp. \$5.00.

This splendid monograph is the result of 5 or 6 years of intensive cooperative research on the structure and function of the hypothalamus carried on in the Institute of Neurology at Northwestern University aided financially by grants from the Rockefeller Foundation. In addition to being a republication of results that have already appeared in numerous technical papers, there is much new material and a more extensive analysis of the literature on diabetes insipidus and closely related subjects.

The method of placing well-circumscribed lesions in rather precise locations within the hypothalamus by inserting a fine unipolar electrode from above with the aid of the Horsley-Clarke stereotaxic instrument, is explained. The anatomy of this part of the brain and of the hypophysis is given in considerable detail. Lesions so placed as to destroy the fibers or the cells of origin of the hypothalamico-hypophyseal tract regularly produce diabetes insipidus. While the symptoms are rather mild in cats, as compared with dogs and rats, the urine output in an average-sized cat may be 1 liter per day. Such operative procedures also result in atrophy of the neural lobe of the hypophysis and retrograde degeneration of the supra-optic nuclei. Several hundred adult cats (eighty-five of which had diabetes insipidus) and eight monkeys (*Macacus mulatta*) were used. Apparently this is the first time diabetes insipidus has been produced experimentally in monkeys.

The onset, development and course of the polyuria and polydipsia in sixty-four of the cats with diabetes insipidus are given in detail with tabulations of individual animals as well as summarizing tables and charts. After an exhaustive analysis of the location of the lesion which produces diabetes insipidus, it is concluded that about half of the fibers of the supra-optico-hypophyseal tract can be interrupted without causing serious disturbances in fluid exchange. A similar consideration is given to the work on the monkeys, two of which developed diabetes insipidus, with results essentially similar to those observed in the cat.

One chapter is devoted to the effects of removal of the neural division of the hypophysis and of merely interrupting the infundibular stem in the cat. It is shown that diabetes insipidus may be produced

providing the removal is complete, i.e., includes the infundibular stem and median eminence of the tuber cinereum, as well as the processus infundibuli. Extirpation of the infundibulum and median eminence only, likewise produces diabetes insipidus. The latter operation also causes atrophy of the processus infundibuli. The presence of a considerable amount of normal anterior lobe substance is necessary for the disorder to occur. On the other hand, lesions in the hypothalamus which do not involve the supra-optico-hypophyseal system do not produce diabetes insipidus.

Various experiments on the effects of water deprivation and administration of pitressin on fluid exchange in cats with diabetes insipidus are detailed. Evidence is presented which indicates that in diabetes insipidus there is a marked decrease or complete cessation of the anti-diuretic principle of the neurohypophysis and that the secretion of the antidiuretic as well as of the pressor and oxytocic incertions are under the control of the hypothalamico-hypophyseal nervous system. Pars intermedia and pars tuberalis are not regarded as elaborators of the posterior lobe hormones. The evidence supporting the various other theories on the nature and cause of diabetes is gone into extensively. Then follow several chapters on various experimental studies on the mechanism of water exchange in cats with diabetes insipidus, which show that thyroidectomy decreases while giving thyroid extract and ingesting salt increase the fluid exchange. Pregnancy and castration have no marked influence on this process; but parturition is seriously disturbed, apparently because of deficiency in the amount of pituitary oxytocic hormone—a problem which needs further investigation.

The final chapter deals with the site of formation of the posterior lobe hormones and includes investigations on the hypothalamico-hypophyseal system in the sperm whale, which has no pars intermedia. The authors conclude that it is the neural lobe, including the median eminence of the tuber cinereum, which is the source of these hormones. They found that pars intermedia undergoes no noticeable histological change and retains its property of expanding melanophores when the hypothalamico-hypophyseal tract is cut, although the neural lobe is atrophic and there is severe diabetes insipidus. Only an occasional nerve fiber enters the intermediate lobe.

Terse summaries at numerous intervals leave no doubt as to the general conclusions drawn. A wealth of accurate morphology backs up the functional implications. Clinical aspects are not neglected.

The book is durably bound. Printing is by photolithography from perfect type-script and is very satisfactory. The seventy-one illustrations are as well reproduced as one would desire. The data are carefully compiled in twenty-six tables. The book closes with a bibliography of 400 references. There is no index, but a detailed table of contents partly serves that purpose. It is invaluable to all who are interested in the hypothalamus and the hypophysis.

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# AN ANATOMICAL STUDY OF THE ROLE OF THE LONG THORACIC NERVE AND THE RELATED SCAPULAR BURSAE IN THE PATHOGENE- SIS OF LOCAL PARALYSIS OF THE SERRATUS ANTERIOR MUSCLE

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## FOUR FIGURES

Because of diverging opinions and conflicting data on the etiology of isolated paralysis of the serratus anterior (magnus) muscle, a study of the origin, structural variations, and relations of the long thoracic nerve was undertaken. A search of the literature revealed only the contributions of Struthers ('07) and Dargent ('35), based on twenty-five dissections and a study of twenty specimens, respectively. The data and conclusions of the present investigation are derived from an analysis of 100 dissections in fifty human cadavera. Many of the structural conditions considered as abnormal and anomalous by Struthers and Dargent are interpreted in the present and larger series as falling within normal variational range.

## COMPOSITION OF THE LONG THORACIC NERVE

The main trunk of the nerve was formed in 84% of the cases by the union of branches from the anterior divisions of the fifth, sixth and seventh cervical nerves. In 8%, the branch from the seventh nerve was lacking, and in another 8%, the nerve was formed from branches of the fifth, sixth, seventh and eighth nerves. The branches forming the long thoracic nerve were noted to arise obliquely from above, downward



and forward in the sagittal plane, and from above, downward and lateralward in the frontal plane (fig. 1). The sixth and seventh branches were usually the largest and approximately of equal size. The branch from the fifth nerve was frequently quite large, especially when it gave off the nerve to the rhomboid muscles. The eighth branch, when present, was always smaller than the others.

In 5% of the dissections, the branch of the fifth nerve coursed independently of the others to be distributed to the

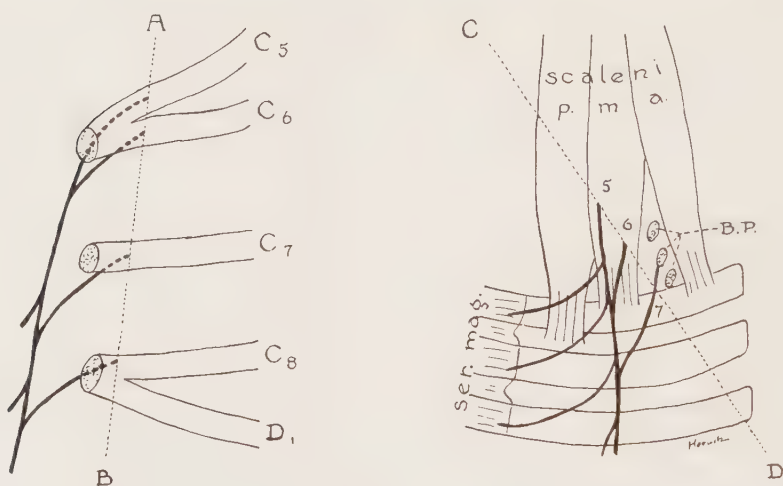


Fig. 1 Formation of the long thoracic nerve from branches of the anterior divisions of the fifth cervical (100%), sixth servical (100%), seventh cervical (92%), and eighth cervical (8%) nerves, as seen in the frontal plane (left illustration) and sagittal plane (right illustration).

upper digitations of the serratus anterior muscle. In a larger number of cases the same arrangement existed, except that the fifth branch sent a small communicating twig to the sixth branch.

In 44% of the specimens, the nerve to the rhomboids arose directly from the branch of the fifth cervical nerve, prior to the latter's exit from the scalenus medius muscle and proximal to its branches to the first digitation of the serratus anterior (magnus) muscle. In the remaining cases, it arose from the anterior division of the fifth cervical nerve directly.

The variations from the usual arrangement, wherein the fifth and sixth branches pierce the scalenus medius, and the seventh and eighth, when present, course between the scalenus medius and scalenus anterior muscles, are represented in the following table:

| <i>Branch of anterior division</i> | <i>Exit in relation to scalenus medius</i> |                |                | <i>Total</i> |
|------------------------------------|--------------------------------------------|----------------|----------------|--------------|
|                                    | <i>Dorsal</i>                              | <i>Through</i> | <i>Ventral</i> |              |
| Fifth cervical nerve               | 2                                          | 84             | 14             | 100          |
| Sixth cervical nerve               | 2                                          | 74             | 24             | 100          |
| Seventh cervical nerve             | 1                                          | 3              | 88             | 92           |
| Eighth cervical nerve              | 0                                          | 0              | 8              | 8            |

In four instances, all branches (fifth, sixth and seventh) pierced the scalenus medius; in twelve, all branches made their exit ventral, and in one, all passed dorsal to this muscle.

#### COURSE OF THE LONG THORACIC NERVE

In 97% of the cases, branches from the fifth and sixth cervical nerves joined before or shortly after their appearance lateral to the scalenus medius muscle, while in 3% this juncture occurred as low as 4 to 5 cm. from the lateral border of the scalenus medius and adjacent to the first digitation of the serratus anterior muscle. This combined trunk then coursed downward and lateralward, across the scalenus posterior muscle to join the branches from the seventh and eighth cervical nerves, when present. The juncture of the fifth, sixth and seventh branches occurred almost immediately in sixty-five specimens; in twenty-two, it took place 2.5 to 5.0 cm. from the lateral border of the scalenus medius, and in thirteen, at or below the clavicle. In one instance the three branches met very low, just above the level of the inferior scapular angle, the independent seventh branch expressly supplying the lower digitations of the serratus anterior.

As the completed nerve dipped beneath the clavicle, at the junction of its medial two-thirds and lateral one-third, it lay 1.5 to 2.0 cm. dorsal to this bone. It passed obliquely downward and lateralward along the chest wall, and became definitely angulated as it coursed over the prominent second rib and its large muscular covering, lying medial to the coracoid

process (fig. 2). Approximately 2.5 cm. below the clavicle it dipped beneath the brachial plexus and the lateral part of the axillary vessels, and coursing beneath the scapula, left the axillary border of the latter 5.0 to 7.5 cm. above the inferior angle. It then followed along the axillary border of the latissimus dorsi muscle, terminating 2 to 3 cm. lateral and anterior to the inferior scapular angle.

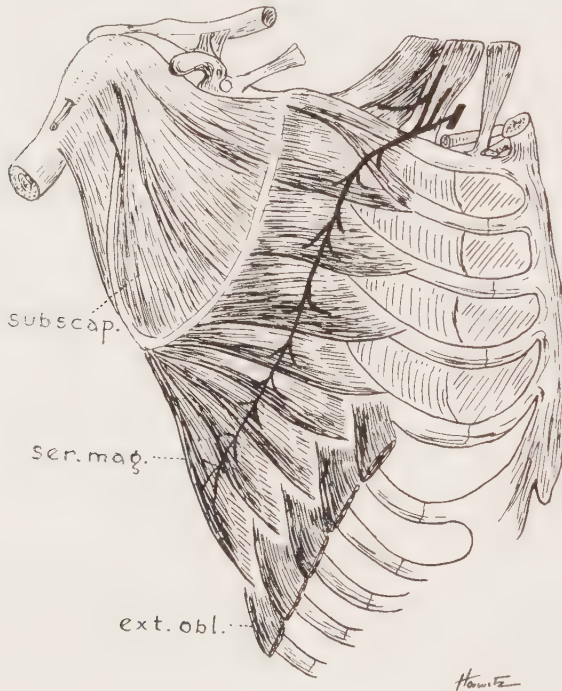


Fig. 2 The course of the long thoracic nerve as revealed by lifting the scapula away from the chest wall. Note the angulation of the nerve over the second rib. Note also the intimate interlacing of the lower digitations of the serratus anterior and the upper digitations of the external oblique muscles.

The nerve supplies two to three branches to each digitation of the serratus magnus muscle, diminishing in size as it progresses distalward so that the branches to the lower digitations are very small. Those to the upper digitations are long and permit considerable play of the long thoracic nerve proximal to the second rib, while the branches of its distal portion are short and restrict its motion.

The findings in the fifty cadavera were almost invariably symmetrical. In eighteen cadavera, the length of the long thoracic nerve was measured from the origin of its fifth branch to its termination. It was longest on the right side and left side in nine specimens each, with a mean length of 28.4 cm. on the right and 28.0 cm. on the left.

#### ACCESSORY INNERVATION OF THE SERRATUS MAGNUS MUSCLE

The possibility of an additional nerve supply from the intercostal nerves may be entertained:

1. When one considers the points of similarity between the serratus anterior muscle and the diaphragm. Both are important respiratory muscles and receive their motor nerves from the neck region. These muscles, originating in pre-muscle masses in the neck region of the early embryo, descend by relative caudal 'migration,' bearing their nerves with them (Keibel and Mall, '10). Threads from the intercostal nerves to the periphery of the diaphragm were first demonstrated by Luschka in 1853, and rediscovered by Cavalie in 1896 (Testut, '11).

2. When one reflects on the apparent insufficiency of the terminal branches of the long thoracic nerve to the lower digitations of the serratus anterior.

3. When one notes how the upper digitations of the external oblique muscle interlace intimately with the lower digitations of the serratus anterior, much as do the diaphragm and transverse abdominalis muscles. The intercostal nerve supply to one might readily send its fibers through to the other as well, and such an arrangement exists in the latter set of muscles.

Branches from the intercostal nerves to the seventh and eighth digitations of the serratus were first noted by one of us in 1931. Dargent ('35) reported his observations, in two out of twenty dissections, of branches from the eighth and ninth intercostal nerves to the lower digitations of the serratus.

In twenty specimens of this series, the serratus anterior was so dissected as to preserve the lateral branches of the anterior divisions of the intercostal nerves as they pierce between its

digitations. Small twigs from these branches were noted to bury themselves and terminate in the muscle bellies of the seventh and eighth digitations, and of the ninth, when present, in every instance, and occasionally in the sixth digitation as well.

Fresh specimens, obtained from six human cadavera shortly after death, revealed the same findings. These nerve filaments were followed to the individual muscle fibers, under strong magnification, and were stained for myoneural endings. Despite repeated efforts and the use of varied techniques (Cajal's reduced silver method VI and Garven's modification of Ranvier's gold chloride method) (McClung, '37), only occasionally were myoneural end plates found. The fact that similar results were obtained in intercostal muscles used as controls, indicates the technical deficiencies of this method of demonstration as a basis for conclusion. While final proof is still sub judice, we believe that the lower digitations of the serratus magnus muscle receive an accessory, albeit inadequate, nerve supply from the adjacent perforating branches of the intercostal nerves.

#### BURSAE RELATED TO THE LONG THORACIC NERVE

Four normal bursae, the subcoracoid, the subscapular, the accessory subscapular and the supracoracoid, as well as adventitial bursae arising through occupational pursuits, may by their proximity affect the long thoracic nerve (fig. 3).

In a study of 100 shoulder joints, the subdeltoid and subacromial bursae were found to be continuous in every instance, incomplete membranous partitions existing in many of the dissections. Thickening and cohesion of the bursal walls in twenty-five instances were interpreted as the effects of previous inflammatory disease. In almost every specimen (all aged over 50 years), some degree of degenerative change was evident in the articular cartilage, capsule, musculo-tendinous cuff or long bicipital tendon (Codman, '34; Meyer, '37). In two instances the subdeltoid bursa communicated directly with the shoulder joint due to complete tears of the supraspinatus tendon.



While the subdeltoid (subacromial) bursa extended frequently for a short distance beneath the coracoid process, a separate subcoracoid bursa was found in eighty-nine cases. In eleven instances, the subdeltoid (subacromial) and subcoracoid bursae communicated by an opening, variable in size, and occasionally merely by a pin-point aperture. In twelve cases, the subcoracoid bursa extended beneath the subdeltoid bursa for a varying distance, although completely separate from it.



Fig. 3 Bursae related directly or indirectly to the long thoracic nerve. 1) Subdeltoid (subacromial), 2) subcoracoid, 3) subscapular, 4) accessory subscapular, and 5) supracoracoid.

The subcoracoid bursa lies between the subscapularis tendon below, and the coracoid process and combined tendon of origin of the short head of the biceps and coracobrachialis muscles above, and has been interposed to permit movement of the former at right angles to the latter during the arc of rotation of the humeral head. It extends posteriorly beneath the coracoid process 1.25 to 1.88 cm. ( $\frac{1}{2}$  to  $\frac{3}{4}$  inch) and about the same distance forward under the combined tendon of the

short bicipital head and coracobrachialis, and medially over the subscapular tendon 0.60 to 1.88 cm. ( $\frac{1}{4}$  to  $\frac{3}{4}$  inch). In two instances this bursa extended 3.75 to 5.00 cm. ( $1\frac{1}{2}$  to 2 inches) medial to the coracoid process over the subscapular tendon,

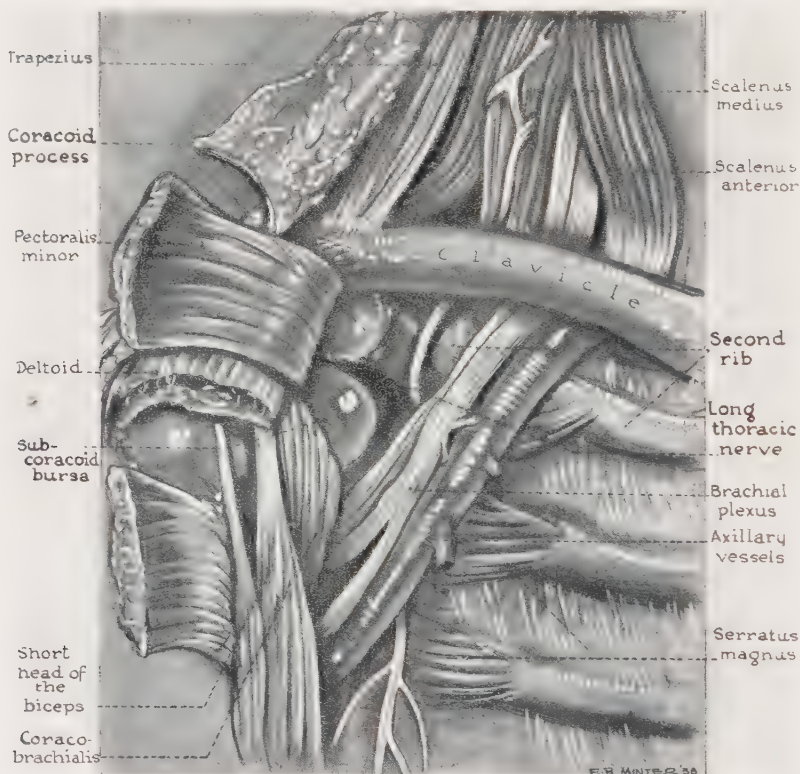


Fig. 4 Illustrating the normal relations of the long thoracic nerve to the coracoid process of the scapula and the second rib, just before the nerve dips dorsal to the brachial plexus and axillary vessels. The walls of the subcoracoid bursa in this specimen were markedly thickened and distended with purulent-like fluid, and the enlarged bursal sac almost contacted the long thoracic nerve. The vulnerability of the nerve to injury and inflammation by its proximity to these structures is apparent.

and in both instances the bursal wall was thickened and distended with fluid as by an inflammatory process (fig. 4). In a third case, a calculus completely filled the bursal cavity. In

each of these three specimens, the subdeltoid (subacromial) bursa was separate and free of involvement.

The subscapular bursa lies between the subscapular tendon and the upper portion of the glenoidal labrum and scapular neck. In seventy-five specimens, the bursa was present in sixty-six, and of the latter, forty-nine communicated with the shoulder joint. The diameter of the bursa varied from 1.25 to 3.75 cm. ( $\frac{1}{2}$  to  $1\frac{1}{2}$  inches). In one instance the bursa projected upward between the superior scapular border and the subscapularis muscle, and protruded medially beneath the coracoid process. It is noteworthy that when several large subscapular bursæ were intentionally distended with fluid, this same course was pursued.

In three instances among seventy-five specimens, a small accessory subscapular bursa was found at the superior border of the scapula, beneath the subscapularis muscle and medial to the main subscapular bursa. In six specimens, small bursæ were present on the superior surface of the coracoid process (supracoracoid), just anterior to the coraco-clavicular ligaments.

#### PRACTICAL CONSIDERATIONS

In view of the many variations in the composition, mode of formation, and relations of the long thoracic nerve, it is easy to understand why identical traumata only occasionally produce an injury to this nerve.

Spasm and rupture of the scalenus medius muscle have been considered as possible etiological factors. It is unlikely that muscular spasm would operate on nerves which always perforate the muscle in the direct course of its fibers. Rupture of the scalenus medius has never been demonstrated, and could be a factor only in those infrequent instances where the nerves, especially that branch from the seventh cervical nerve which supplies the strongest (inferior) portion of the serratus anterior muscle, pierce the scalenus medius.

Factors to be operative would more likely involve the nerve after it has been formed completely. The occurrence of this

paralysis following sudden direct stress to the shoulder and scapular regions, or after prolonged, heavy weight bearing, has frequently been noted. In the cadaver the nerve is seen to become deflected in its course and angulated over the second rib. This angulation becomes exaggerated and the nerve is made tense by downward thrust of the shoulder girdle, or by lateral tilt of the head and neck in the opposite direction.

In addition, the nerve as it courses between the second rib and its large muscular digitation, medially, and the coracoid process, laterally, is susceptible to compression when lateral pressure is exerted against the scapula. This liability to compression is enhanced when the nerve lies adjacent the bared base of the coracoid process, a position existing when the branches of origin of this nerve pierce the posterior part of the scalenus medius or course between the scalenus medius and scalenus posterior.

From our study it is also apparent that certain adjacent bursae, notably the subcoracoid and subscapular, enlarged by trauma or by inflammation, may exert a direct mechanical (pressure) effect upon the nerve, or affect it by the contiguous spread of inflammation. The occurrence of paralysis of the long thoracic nerve in the course of infections, such as influenza, pneumonia, typhoid fever, puerperal sepsis, etc., and the evidences of localized tenderness, swelling and heat in the region of the scapular bursae, emphasize the significance of the infectious factor.

Compression of the nerve might readily be initiated or perpetuated by the supine position, as in prolonged labor, tedious and long surgical operations, and in patients bedridden for long periods. The clinical experience of exaggeration of pain by nocturnal compression in cases of isolated paralysis of the serratus anterior muscle, and its relief by arising or by sleeping in the prone posture, as reported by us in a separate communication, is in keeping with such a concept. This factor probably acts only if the nerve is susceptible because of neighboring or systemic infection, or irritated by excess activity of the serratus anterior muscle. Like musculospiral paralysis,



it is a condition evolved by a combination of factors, viz., individual, anatomical and postural, and an organism susceptible because of disease or trauma.

It is likely that transient compression of the long thoracic nerve is not infrequent, as is evidenced by the complaint of patients experiencing difficulty in raising their arms in the morning as for combing or shaving, this incapacity disappearing after an hour or so of the upright position.

#### SUMMARY

One hundred dissections in fifty cadavera were made to determine the composition, course, relations and other characteristics of the long thoracic nerve. From this study, and in view of the comparative rarity of its paralysis, it is felt that localized paralysis of the serratus anterior (magnus) muscle is an outgrowth of the action of certain injurious agents, in the presence of a favorable morphological arrangement of the long thoracic nerve and its surrounding parts.

#### ACKNOWLEDGMENT

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## AFFERENT INNERVATION OF THE SKIN

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FIVE FIGURES

The peripheral branches of the cutaneous nerves form a plexus in the subcutaneous tissue and secondary plexuses in the corium, particularly at the boundary between the reticular and papillary layers and in the subepithelial zone. These nerve plexuses are associated with the corresponding subcutaneous and cutaneous vascular plexuses. The cutaneous nerves include all afferent fibers which terminate in the skin and the sympathetic fibers through which the cutaneous vessels, sweat glands, erector pili muscles, etc., receive their efferent innervation. The afferent supply includes both myelinated and unmyelinated fibers; the sympathetic fibers are mainly unmyelinated.

Terminal arborizations of afferent nerve fibers in the deeper layers of the epidermis have been described repeatedly. The arrangement of afferent fibers in relation to the hair follicles also has been described in minute detail. Encapsulated receptors have been amply demonstrated in the dermal papillae in certain areas of the skin and in the subcutaneous tissue, but these organs are not sufficiently abundant and widespread to play a major role in cutaneous sensitivity. Afferent nerve fibers undoubtedly terminate in relation to cutaneous blood vessels and in the connective tissue of the corium, but little is known regarding the exact morphological characters and relationships of the terminal structures associated with these fibers.

Current descriptions of intraepidermal nerve fibers are based mainly on preparations of skin taken from the snouts and muzzles of mammals and the fingers of man, i.e., regions of the skin in which the sensory innervation is particularly abundant. Intraepidermal nerves in the human skin, except that of the hands and certain other highly sensitive areas, have been described by relatively few investigators. Those who have investigated the afferent innervation of less sensitive cutaneous areas have emphasized the difficulty in demonstrating intraepidermal fibers and the relative scarcity of nerve fibers in the epidermis. According to certain investigators intraepidermal nerve fibers may be demonstrated more readily in preparations of fetal skin than in those of adult skin. Certain authors have pointed out, however, that intraepidermal nerve fibers are more abundant in the skin of the human fetus than in that of the adult.

The sensory innervation of every hair follicle involves a variable number of afferent nerve fibers, but some of the follicles are surrounded by much more abundant nerve fiber investments than others. The afferent fibers which terminate in relation to hair follicles exhibit essentially the same morphological characters as those whose terminal branches penetrate the epidermis. They probably belong to the same functional category.

Nerve fiber terminations in the subepidermal connective tissue have been described particularly by Woollard ('36, '37). According to his account, the terminal branches most commonly grow finer and finer until eventually they "pass as it were beyond the range of vision." The precise position of their terminal ends, consequently, cannot be determined with certainty. They probably are most abundant in the zone immediately beneath the epidermis.

Nerve fibers commonly are present along the cutaneous blood vessels, including the arterioles and venules and many of the capillaries, throughout the corium. Many of these fibers obviously are sympathetic; others afferent. Some of the latter probably terminate in relation to the vessel walls,

but little is known regarding their precise modes of termination.

All components of the cutaneous nerves probably take part in the formation of the plexuses in the corium. These plexuses probably are present all over the body but are richer in nerve fibers in the more sensitive areas than in the less sensitive. The subepidermal plexus may be regarded as essentially terminal. It consists of an irregular meshwork of minute nerve fiber bundles in the zone immediately subjacent to the epidermis. The finest fibers in this plexus, according to Woollard ('37), join and fuse with one another so that there is formed a continuous neurofibril reticulum. The final endings in the epidermis and the subepidermal zone in general emerge from the subepidermal plexus.

#### MATERIAL AND METHODS

The primary objectives of the present investigation have been more exact data regarding the relationships of afferent nerve fibers to the cutaneous blood vessels and the modes of termination of afferent fibers in the connective tissue of the corium. Preparations of both human and animal skin have been used. In order to obtain preparations devoid of efferent nerve fibers, skin was taken from the paw pads and other parts of the forelimbs of cats in which the sympathetic fibers in the forelimb had undergone complete degeneration following extirpation of the inferior cervical ganglion and the first and second thoracic segments of the sympathetic trunk. Various technics have been employed, including modifications of Bielchowsky's and Cajal's silver impregnation methods and Bodian's protargol method. The most satisfactory preparations have been obtained by the use of a modification of Bodian's technic.

#### ANATOMICAL DATA

In sections of skin, either human or animal, taken at right angles to the surface, in which the nerve fibers are successfully impregnated, the nerve plexuses associated with the

vascular plexuses stand out prominently. From the plexus at the border of the reticular layer of the corium with the papillary layer, i.e., the subpapillary plexus, offsets extend into both layers. Nerve fiber bundles of various sizes also extend along the vessels, particularly the arteries and arterioles, which approach the epidermis. In preparations of skin in which the subepidermal papillae are well developed, nerve fibers extend along the arterial limbs of the vascular loops into the apices of the papillae. Some of the finer unmyelinated fibers obviously terminate in relation to the vascular musculature; others probably do not. Of the myelinated fibers associated with the vascular loops, some terminate in relation to the vessels; some join the subepidermal plexus, and some penetrate the deeper layers of the epidermis. In preparations of skin in which subepidermal papillae are absent, or only poorly developed, nerve fibers likewise extend along the arterial vessels toward the epidermis. Some of these terminate in relation to the vessels, including the capillaries; some join the subepidermal plexus, and some penetrate the deeper layers of the epidermis. In general the cutaneous innervation is less abundant in areas in which subepidermal papillae are absent, or only poorly developed, than in areas in which papillae are well developed.

Preparations of the skin of the paw pads and other parts of the forelimbs of cats in which the sympathetic nerves of the forelimb had undergone degeneration exhibit essentially the same distribution of the cutaneous nerves as preparations of normally innervated skin, but the finer unmyelinated fibers which terminate in relation to the vascular musculature, the erector pili muscles, etc., are absent. In the absence of sympathetic fibers, all the nerve fibers present in the skin must be regarded as components of the posterior, or sensory, nerve roots. Most of these are myelinated, but some appear to be unmyelinated.

Afferent nerve fibers which terminate in relation to hair follicles approach the follicles from all sides as well as from deeper levels. They form an investment around the follicles



in which the fibers are more or less regularly arranged and from which terminal branches extend into the outer root sheath where they end in disc-like expansions. According to Woollard ('37), five or six nerve fibers commonly enter the plexiform investment of a human hair follicle. In the cat's forelimb, as indicated by our preparations, some follicles seem to be innervated through a larger number of afferent fibers. The arrangement of the nerve fibers in the peri-follicular investments conforms in all essential respects to the current descriptions of the perifollicular investments in the human skin.

Some afferent fibers derived from the subepidermal and deeper cutaneous plexuses terminate in the connective tissue adjacent to these plexuses. Of these, some, as has been pointed out by Woollard ('37), probably end without specialized terminal structures. Some end in relatively simple terminal arborizations; some in delicate lamellated end organs (fig. 1).

The lamellated end organs observed in our preparations are slender elongated bodies with a delicate central core which is traversed by a nerve fiber and surrounded by few delicate lamellae and a delicate connective tissue capsule. Between the superficial lamella and the capsule is a relatively wide lymph sinus (figs. 1 and 2). These organs commonly are located in proximity to blood vessels and in intimate relation to adjacent lymph channels. Their peripheral lymph sinuses probably are connected with adjacent lymphatics. These bodies are not structurally identical with any of the other encapsulated end organs known to exist in circumscribed areas of the skin.

End organs structurally similar to the lamellated bodies described above have been described by Boeke ('33) in the papillary layer of the corium in the snout of the mole (*Talpa europaea*). They are more abundant in our preparation of skin taken from the cat's forelimb than in those of human skin. We have observed them only in the papillary layer of the corium. Their distribution probably is not limited to

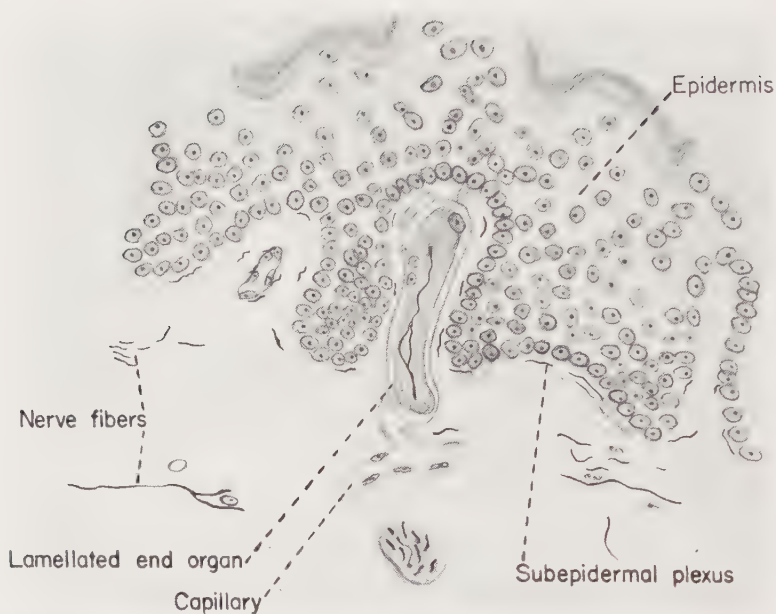


Fig.1 From a section of the skin of the paw pad of the forelimb of a cat in which the sympathetic nerve fibers had undergone degeneration following extirpation of the inferior cervical ganglion and the first and second thoracic segments of the sympathetic trunk.



Fig.2 Lamellated end organ in the papillary layer of the corium from the leg of the cat.

Fig.3 Lamellated end organ in the corium of human skin from the sternal area.

restricted cutaneous areas, since they have been observed in preparations of human skin taken from the anterior aspect of the thorax (fig. 3) as well as in preparations of skin from



Fig. 4 From a section of the skin of the paw pad of the forelimb of a cat in which the sympathetic nerve fibers had undergone degeneration following sympathetic ganglionectomy.

more sensitive areas, but they are not equally abundant in all cutaneous areas. Many of our preparations of human skin, taken from various parts of the body, exhibit no structures which could be identified as lamellated end organs.

The afferent nerve fibers associated with the blood vessels in the papillary layer of the corium, including the capillaries, are derived mainly from the plexus at the border of the reticular layer with the papillary layer. In sections of skin of the sympathectomized forelimb of the cat, bundles consisting of relatively few fibers accompany the vessels toward the epidermis. In areas in which the subepidermal papillae are well developed, e.g., the paw pads, the nerve fiber bundles

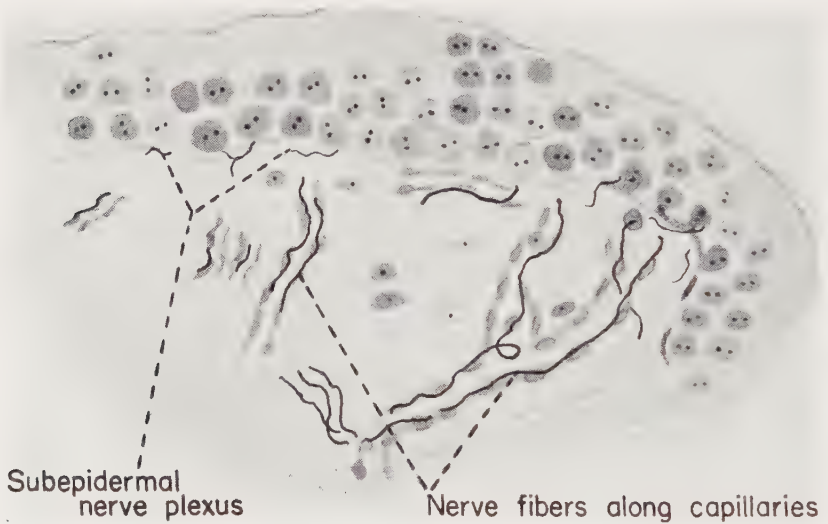


Fig. 5 From a section of the skin of the foreleg of a cat in which the sympathetic nerve fibers had undergone degeneration following sympathetic ganglionectomy.

extend into the papillae mainly along the arterial limbs of the vascular loops (fig. 4). Some of these fibers terminate in the vessel walls or adjacent to them; some join the subepidermal plexus, and some penetrate the epidermis where their terminal branches end in relation to cells in the deeper epidermal layers. In the absence of subepidermal papillae, afferent nerve fibers are related to the vessels in the superficial layer of the corium in essentially the same manner as to the papillary vessels (fig. 5).

The subepidermal plexus comprises mainly afferent fibers. Some sympathetic fibers probably join this plexus, but it is not materially altered by degeneration of the sympathetic fibers following sympathectomy (fig. 5). Many of the fibers which extend into the epidermis may be traced directly from this plexus, particularly in areas in which subepidermal papillae are absent or only poorly developed. Fibers derived from this plexus also terminate in relation to hair follicles and in the adjacent connective tissue. Fusion of nerve fibers in the subepithelial plexus, as reported by Woollard ('37), has not been observed in our preparations. This negative observation, however, should not be regarded as incompatible with the positive finding of Woollard in whole mounts of the skin, since sections exhibit only fragments of the plexus.

#### COMMENT

The results of the present investigation support the assumption that afferent nerve fibers terminate in the deeper layers of the epidermis and throughout the corium. Intraepidermal nerve fiber terminations probably are less abundant, particularly in the less sensitive areas of the skin, than current accounts seem to indicate. The subepidermal plexus is made up of relatively slender strands of nerve fibers, but must be regarded as an important feature of the cutaneous nerve supply both structurally and functionally.

The afferent nerve fibers within the skin vary in caliber within a relatively wide range, but do not fall into clear-cut morphological classes according to size. Afferent fibers of the largest calibers in the posterior nerve roots probably do not extend into the skin. Our observations on the intraepidermal nerve fibers corroborate those of earlier investigators which indicate that these fibers are the terminal branches of myelinated fibers of relatively large caliber. The nerves most intimately associated with the blood vessels in the corium include afferent fibers of small caliber many of which undoubtedly terminate in intimate relation to the smaller vessels. The fibers which terminate in the lamellated end



organs in the papillary layer of the corium, observed in our preparations, are mainly of medium and small caliber. Our observations do not support the assumption that afferent fibers of large calibers terminate in the deeper layers of the corium except in relation to hair follicles.

On the basis of extensive data obtained by experimentation involving stimulation, including cutting, of the epidermis, Waterston ('33 a, b) advanced the theory that the intraepidermal nerve fibers subserve the sense of touch, but play no part in sensations of pain or pressure. Conduction of tactile impulses by afferent fibers falling within the caliber range of those whose terminal branches penetrate the epidermis and impulses of pain by fibers falling within the caliber range of the afferent fibers which terminate in relation to the smaller cutaneous blood vessels also has been demonstrated by means of the cathode ray oscillograph (Heinbecker, Bishop and O'Leary, '32). The functional specificity of all afferent cutaneous nerve fibers, however, cannot be regarded as experimentally demonstrated. Certain experimental data, moreover, support the conclusion that impulses of touch, pressure and pain may be conducted by the same peripheral afferent fibers, or fibers belonging to the same morphological category, particularly from the central area of the cornea (Nafe and Wagoner, '37 a, b, c).

In addition to the vascular changes in extensive areas of the skin resulting from localized thermal stimulation, local cooling of the skin elicits reflex constriction and local warming, within certain limits, elicits reflex dilatation of the cutaneous vessels in the localized areas in question. These changes normally are accompanied by sensations of cold and warmth respectively. The peripheral thermal apparatus, therefore, obviously is intimately associated with the cutaneous blood vessels. The afferent conduction pathways for thermal impulses arising in the skin undoubtedly include fibers which terminate in relation to the smaller vessels, particularly in the papillary layer of the corium. Experimental data reported

by Nafe and Wagoner ('37 a, b, c) also support the assumption that the generation of thermal impulses in the skin is correlated with caliber changes in the superficial vessels.

Although the assumption that tonic changes in the cutaneous blood vessels play a causative role in the generation of thermal impulses implies that all sensations of warmth are accompanied by vasodilatation and all sensations of cold by vasoconstriction, it does not necessarily imply that all changes in vascular tonus must be accompanied by thermal sensations. In some instances, as shown by the experimental records of Nafe and Wagoner, in which the change in vascular tonus took place very slowly or was small in extent no conscious experience was reported. These investigators also have pointed out that under conditions of complete adaptation, i.e., with cessation of actual change in the tonic state of the vascular musculature, sensations of warmth or cold subside. These facts are not incompatible with the theory that sensations result from nerve excitation in which changes in vascular tonus are causative factors.

Exaggerated thermal stimuli, either hot or cold, not uncommonly elicit sensations of pain. Pain elicited by heat, in the absence of tissue damage, probably results from spastic contraction of blood vessels caused by heat stimulation beyond the range in which the vessels respond by dilatation. Pain elicited by cold stimulation also is associated with extreme vasoconstriction. The facts that thermal and painful sensations in general are accompanied by appropriate changes in vascular tonus, according to Nafe and Wagoner, is incompatible with the theory that the receptors and the peripheral afferent nerve fibers involved are specific for single qualities of sensation or single types of stimulation.

The flow of blood in individual cutaneous capillaries frequently is intermittent. Furthermore, under normal conditions, not all the capillaries present are functionally patent at the same time. On the basis of extensive observations on the skin of human subjects, Bordley et al. ('38) have advanced the theory that individual capillaries are endowed

with occlusive mechanisms by means of which the intermittent flow is brought about. If the assumption that the generation of impulses of warmth and cold in the skin is correlated with vasodilatation and vasoconstriction is correct, it seems not improbable that the distribution of warm and cold spots also is correlated with the tonus of the peripheral vessels. The minute areas which are sensitive to warm stimuli probably are those in which the capillaries are responsive, at the moment, to impulses which normally elicit dilatation whereas those which are sensitive to cold stimuli are the areas in which, at the moment, the capillaries respond most readily to constrictor impulses. The constant shifting of the pattern of the warm and cold spots probably could be explained most satisfactorily on this basis.

Certain data advanced by Lewis ('37) seem to indicate that not all posterior root fibers are essentially sensory. In many normal subjects a widespread area of hyperalgesia appears around a point of injury or faradic stimulation of the skin which lasts for several or many hours and may be accompanied by mild smarting of the skin. Similar hyperalgesia may be produced by stimulating a cutaneous nerve. This effect is not prevented by blocking the nerve proximal to the point of stimulation, but is prevented by blocking the nerve distal to the point of stimulation. The flare due to dilatation of the cutaneous vessels around a point of injury to the skin also involves the action of posterior root fibers, since it fails to appear in an area in which the posterior root components of the cutaneous nerves have undergone degeneration, but is not affected by degeneration of the sympathetic fibers. Stimulation of posterior nerve roots furthermore results in localized peripheral vasodilatation. The reactions enumerated above clearly are mediated through identical or closely related neural mechanisms made up of the peripheral portions of posterior root components of the cutaneous nerves. According to Lewis, they involve a branching system of axons which is incompatible with sensory localization. The functioning of these mechanisms undoubtedly involves the liberation of potent substances in the skin. The flare, for example,

is known to involve the release of a histamine-like substance. The release of chemical substances in areas of hyperalgesia also has been demonstrated. Since the reactions in question are essentially protective, Lewis has designated the neural mechanisms through which they are mediated the 'nocifencer' system.

The data obtained in the present investigation afford no direct evidence of the existence in the skin of an independent system of branching axons of posterior nerve root origin such as Lewis has postulated, but the existence of posterior root fibers which are not essentially sensory in function is not precluded. On the other hand, the fact that appropriate stimulation of certain posterior root fibers results in tissue responses at the periphery does not prove the incapacity of these fibers to function as afferent conductors.

The extent of the terminal branching of posterior root fibers which join the subepidermal plexus could not be determined in our preparations. If this plexus includes fibers which fuse with one another, as has been observed by Woollard, it might afford the anatomical structure through which impulses resulting from the liberation of appropriate chemical substances in the skin spread into adjacent areas. The phenomena associated with hyperalgesia around a point of injury in the skin or due to nerve stimulation, the flare, and localized vasodilatation due to stimulation of posterior root fibers probably could be explained on this basis without the assumption of a special system of posterior root fibers. The lamellated end organs located in the corium, by virtue of their intimate relationship to the smaller blood vessels and adjacent lymphatics, might conceivably play a role in this function as well as in the responses to thermal and painful stimulation.

#### SUMMARY

The distribution of nerve fibers in the skin and their modes of termination have been studied in preparations of normal human and animal (cat) skin and skin of the forelimbs of cats in which the sympathetic fibers had undergone degeneration.

The data obtained support the accepted views according to which afferent fibers terminate in the deeper layers of the epidermis and throughout the corium, and that the intra-epidermal fiber terminations, like those associated with the hair follicles, are connected with myelinated fibers of relatively large caliber.

Afferent fibers are intimately associated with the smaller blood vessels in the corium, including the capillaries in the papillary layer. Many of these terminate in intimate relationship to the vessels. Lamellated end organs are described both in the skin of the cat's forelimb and in human skin, particularly in the papillary layer of the corium, which are structurally similar to end organs described by Boeke in the corium of the snout of the mole.

The probable functional relationships of afferent components of the cutaneous nerves are discussed in the light of recently reported experimental studies.

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# FASCIAL CONTINUITIES IN THE ABDOMINAL, PERINEAL, AND FEMORAL REGIONS <sup>1</sup>

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TWO PLATES (FOUR FIGURES)

In descriptions of the fascial layers of the human body, so complete has been the regional bias that each area has usually been discussed with little regard to the continuities of the layers therein; emphasis has been placed on minutiae of local structure, broader relationships disregarded.

In a recent study of fascial strata, the writers have placed the consideration of continuity before that of area. That portion of the study which we here report concerns the fascia of the perineum primarily, in its relation to strata of adjacent portions of the abdomen and the thigh. Our descriptions are based upon careful dissections of four specially selected female bodies.<sup>2</sup> In the four bodies, the anatomical features with which we are here concerned were, in every important respect, similar; our comments therefore refer to all.

The tela subcutanea of the lower abdomen is regularly described as two-layered; beneath the deeper of the two strata,

<sup>1</sup> Contribution no. 264 from the Anatomical Laboratory of Northwestern University Medical School.

<sup>2</sup> Concerning which the following data may be of interest:

| <i>Specimen</i> | <i>Race</i> | <i>Age</i><br>years | <i>Weight</i><br>pounds | <i>Height</i><br>inches |
|-----------------|-------------|---------------------|-------------------------|-------------------------|
| I               | Negro       | 55                  | 126                     | 65                      |
| II              | Negro       | 40                  | 70                      | 64                      |
| III             | Negro       | 35                  | 95                      | 64                      |
| IV              | Negro       | 25                  | 70                      | 67                      |

The four drawings were prepared from specimen IV.

according to some authorities, a third layer occurs. In our specimens, external to the musculature, these three layers of fascia are readily distinguishable; they are, from without inward, the superficial layer of the superficial fascia (fascia of Camper), the deep layer of the superficial fascia (fascia of Scarpa), the deep fascia (innominate fascia of Gallaudet).

In textbooks of anatomy the two layers of superficial fascia are said to continue, separate and without interruption, into the urogenital portion of the perineum; on the fate of the deep fascia, no comment is made. But in Gallaudet's excellent treatise the latter is described as having a perineal homologue. In our dissections the superficial layer of the superficial fascia, i.e., the fatty stratum which forms a general subcutaneous coating, is traceable, without pronounced local modification in character, from the abdomen to the perineum and thigh (fig. 1); in the anal part of the perineum it is increased in bulk, filling the ischiorectal fossa (partially enucleated therefrom in left half of fig. 1).

The deep layer of the superficial fascia is membranous, not fatty, in character, and in one of the four specimens, divisible into two leaves over the inguinal part of the abdomen (fig. 1). It is continued without interruption onto the thigh and into the perineum (lifted by probe in fig. 1), but fastens locally along the inguinal ligament. On the thigh (at the artificial hiatus containing probe in fig. 1) the layer covers the fascia lata; in the urogenital part of the perineum, as the fascia of Colles, it overlies the inferior perineal fascia and is attached laterally to a fibrocartilaginous ridge on the ischiopubic rami; posteriorly it unites with the inferior fascia of the urogenital diaphragm and then passes into the ischiorectal fossa. As pointed out by Gallaudet, in the latter situation this layer reduplicates the obturator fascia and the inferior fascia of the pelvic diaphragm. Over the gluteus maximus muscle posteriorly the layer ceases to be distinguishable from the fatty pannicle. In the greater lip of the pudendum the layer is somewhat elevated over the bulb of the vestibule (incised

in fig. 1 to show the deep fascial investment of the bulbocavernosus muscle within the superficial perineal compartment).

In our four specimens a diverticular process of the deep layer of superficial fascia occurred as a downward extension into the perineum from the subcutaneous inguinal ring (lifted upward and lateralward in fig. 1., replaced in natural position in fig. 2).<sup>3</sup> Lateral to the body of the clitoris and bulb of the vestibule, it is prolonged posteriorly to a point opposite the urethral orifice. The diverticulum is entirely separate from the overlying fatty pannicle and underlying Colle's fascia, and contributes appreciably to the bulk of the greater labium. In two well-injected specimens blood vessels were found within the substance of the diverticulum; derived from the deeper strata, they may be regarded as comparable to the external spermatic vessels of the male. Within the diverticulum is contained the round ligament, ensheathed, successively, by fasciae continuous with the abdominal layers (figs. 3 and 4; diverticulum transected); the entire assemblage of structures therefore bears some resemblance to the spermatic cord in the male.

The innominate fascia of the abdomen covers the external surface of the external oblique aponeurosis and upon reaching the free edge of the inguinal ligament is carried inferiorly onto the thigh as the fascia lata. The continuity with the homologous layer in the perineum, the inferior perineal fascia (Gallaudet, '31), may be demonstrated in two ways: first, by following the innominate fascia into the perineum just lateral to the pubic symphysis (fig. 3, medial dotted line; fig. 4, corresponding incision); and secondly, by following the fascia lata medially, where the layer is fused to the periosteum of the ischiopubic ramus, then continuous with the inferior perineal fascia (fig. 3, lateral dotted line; fig. 4, corresponding incision).

<sup>3</sup> Piersol apparently refers to this structure in describing a discrete, fascia-invested pad of fat in the greater labia, continuous with the fat of the preperitoneal connective tissue; but he does not describe its diverticular character, or account for its fascial continuities.

Thus it may be seen that the inferior perineal fascia, a true muscle fascia, bears the same relationship to the superficial perineal muscles as the innominate fascia does to the external oblique muscle and its aponeurosis or as the fascia lata does to the femoral muscles. This general deep fascial stratum is covered externally by the deep layer of superficial fascia discussed above.

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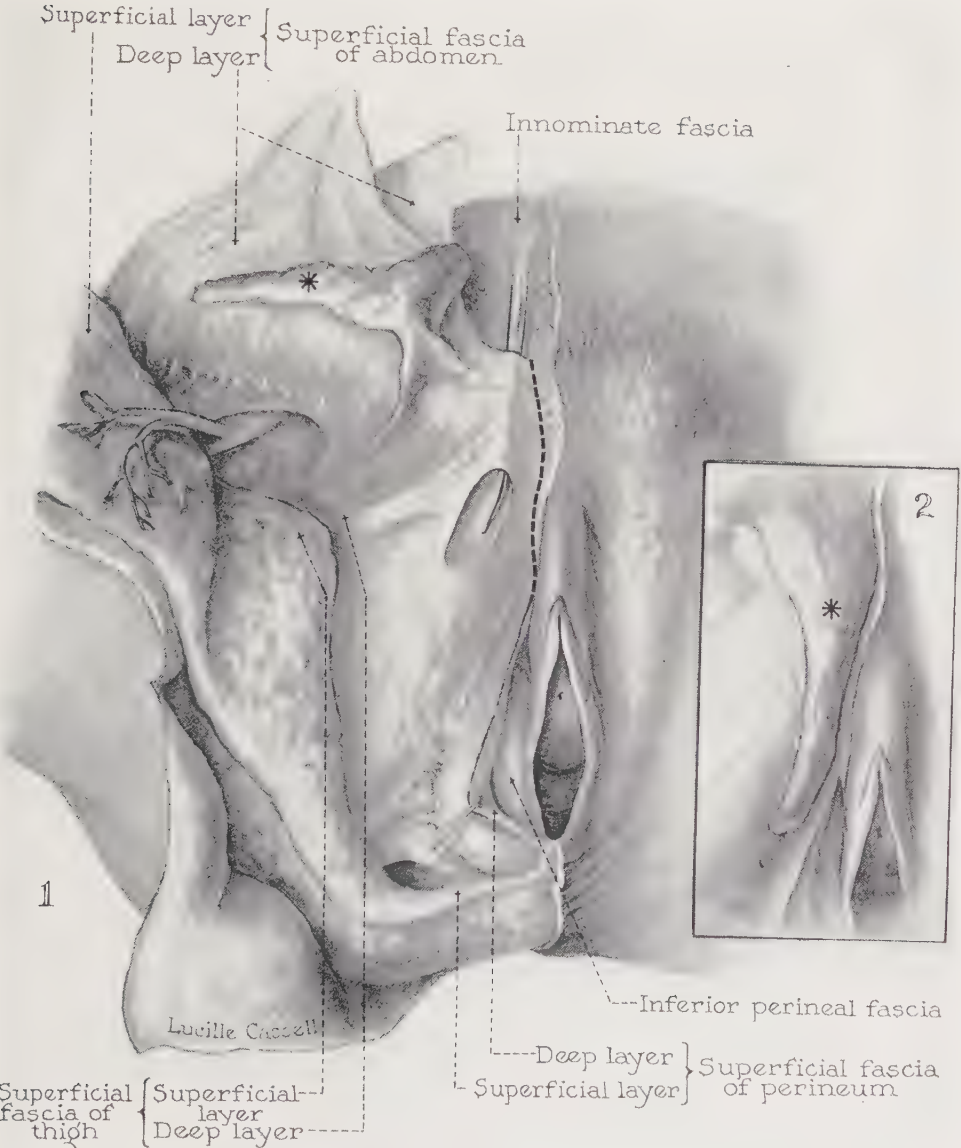
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#### PLATE 1

##### EXPLANATION OF FIGURES

1 The superficial layer of the superficial abdominal fascia has been freed and turned aside, the deep layer of the same stratum elevated to show the deep fascia (innominate fascia of Gallaudet). The dotted line indicates the direction in which the incision is extended in figure 3 to expose the deep or muscle fascia. From the subcutaneous inguinal ring a diverticular process (marked \*) of the deep layer of superficial fascia, containing the round ligament, extends into the perineum, contributing to the tissue within the greater lip. Through the artificial hiatus (at probe) in the deep layer is seen the fascia lata of the thigh. In the perineum, as the fascia of Colles, it is attached to the ischiopubic rami. By opening this layer, the superficial perineal interspace is revealed, within which is seen the inferior perineal fascia as it invests the bulbocavernosus muscle.  $\frac{2}{3}$  natural size.

2 The diverticular process replaced in its natural position along the body of the clitoris and the bulb of the vestibule.





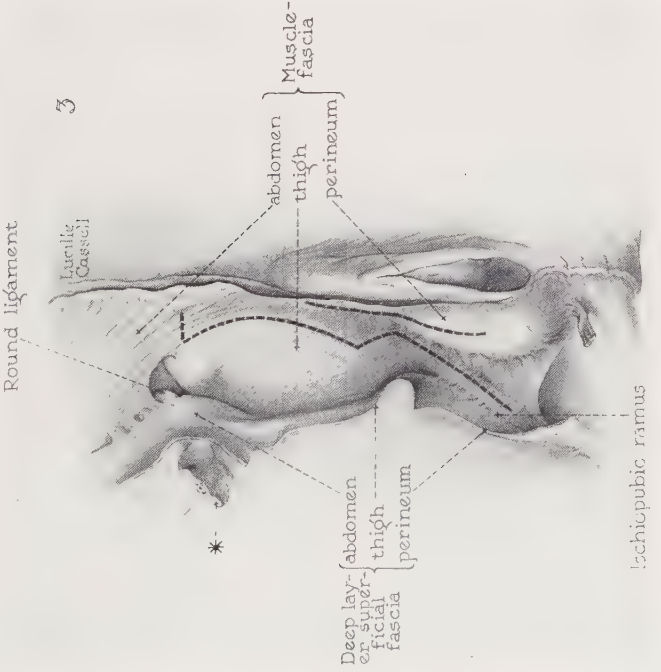
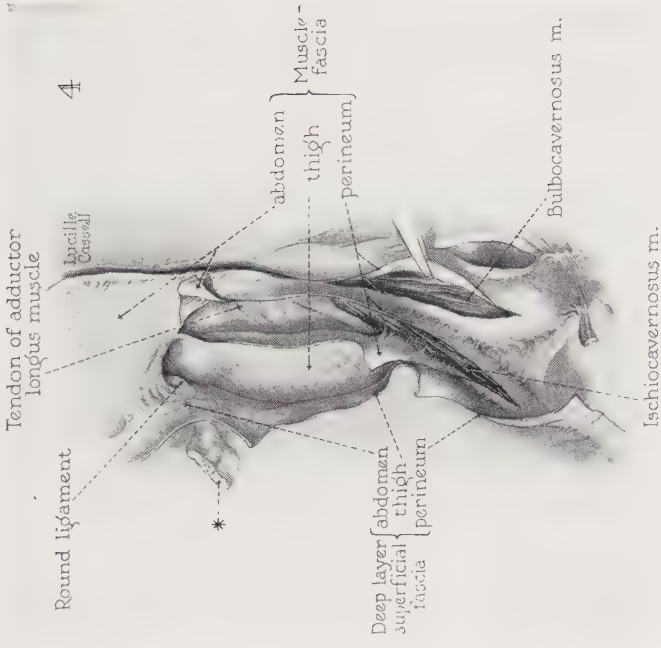
## PLATE 2

### EXPLANATION OF FIGURES

3 The continuity of abdominal and perineal fascial layers, continued. The integument, intact on the right has been removed on the left, together with the superficial, fatty, layer of fascia. The deep, membranous, layer of fascia has been reflected on the abdomen to expose the innominate fascia and subcutaneous inguinal ring, on the thigh to expose the fascia lata, and in the perineum cut away near the attachment to the ischiopubic ramus to expose the inferior perineal fascia. The broken lines indicate the course of the incisions followed to demonstrate the features seen in the succeeding figure.  $\frac{1}{2}$  natural size.

4 Incisions, made in the inferior perineal fascia, along the lines indicated in figure 3, reveal the continuity of the deep fascia from the abdomen and thigh to the urogenital part of the perineum. The fascia in turn has been opened to show the superficial perineal muscles for which it forms the intimate fascial investment.  $\frac{1}{2}$  natural size.

FASCIAL LAYERS OF THE HUMAN BODY  
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# THE INFLUENCE OF A NORMAL OVARY ON THE FORMATION OF TYPICAL IRRADIATION TISSUES IN THE X-RAYED OVARY

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When both ovaries of sexually mature guinea pigs were treated with a minimum sterilization dose of x-rays (2160 r units) the normal ovarian stroma was replaced by masses of glandular cells resembling young luteal cells (interstitial gland), formed from follicles of all sizes. No further normal growth of follicles occurred and no corpora lutea were formed. This ovarian change was associated with irregular oestrous cycles, prolonged oestrous openings in which cornification or all types of epithelial cells of oestrus were excessive, and great proliferation of the mammary gland (Genther, '31, '34).

We attempted to produce glandular tissue in a single ovary to determine the effects of such tissue on the functioning of the remaining normal ovary.

## METHODS AND RESULTS

Twelve virgin sexually mature guinea pigs were irradiated as described previously ('34), the body being protected so that only the region over one ovary was exposed to the x-rays.

In eleven animals there was no delay in the appearance of the first oestrus following the irradiation, as occurred frequently when both ovaries had been irradiated. The cycles thereafter were very regular with normal typical oestrous openings. Only one animal had an initial delay of 38 days, followed by one normal cycle, then a 13-day cycle with an oestrous opening of 13 days with excess cornification in the vaginal smears, followed again by regular and normal cycles.

The reproductive processes seemed somewhat impaired in spite of the normal cycles. Three animals apparently did not mate with the males which had been placed in their cages 2 days before the anticipated oestrous openings, for no sperm were found in the smears nor did pregnancies result. Two others became pregnant but aborted at 33 and 35 days. One gave birth to two normal healthy young but was ill thereafter and died 25 days after parturition. Matings were not attempted in the other six animals.

The untreated ovaries of each pair were not hypertrophied. Microscopically all were normal in structure. Six contained three corpora lutea each, three had two each, three had one each at the time of death.

All the irradiated ovaries were similar in structure. They were small and atrophic, like ovaries which had received a larger more severe dose of x-rays (Genther, '31). The stroma was compact. One ovary at 58 days and another at 65 days after irradiation had quite a number of primordial follicles though nowhere near the normal number, one small growing follicle each, and a great many small cavities with zona pellucida remnants. But all other ovaries from about 3 to 8 months after irradiation were practically devoid of follicles, both normal and atretic, or had only a few primordial follicles. No corpora lutea were present, only pre-irradiation scars in some, showing that follicular development was suppressed immediately after irradiation. Small cysts, none much larger than a well-developed corpus luteum, were present in five ovaries. In the two ovaries cited above, small cysts were seen forming from degenerating follicles.

In four of the twelve ovaries portions of the cortical stroma looked slightly glandular in that cells were somewhat enlarged; but they resembled normal stroma cells more closely than glandular tissue. In three of these four animals, the nipples had been noticeably elongated, and in two of these there was some proliferation of mammary tissue, more than usually during a normal oestrus. But there was no disturbance in the cycles at any time, with the one exception mentioned; this latter animal showed no mammary growth.



These slight disturbances may have been due to some unknown factor.

The hypophyses were slightly larger (heavier) than those of untreated virgins of the same weight. The hypophysis of each of seven animals was implanted into a normal immature female guinea pig. Each recipient reacted exactly as did those which had received an implant from a normal female guinea pig (Schmidt, '37) from a corresponding stage of the oestrous cycle.

In conclusion, the presence of the normal ovary apparently prevented the formation of the glandular tissue in the irradiated ovary. The active corpora lutea may have been responsible for this. They inhibit the complete development of follicles in normal ovaries, and may therefore prevent the formation of the glandular tissue which is of follicular origin. The formation of glandular tissue may be a compensatory reaction, an attempt to produce the hormone which the follicles normally produce; in the presence of a normal ovary such compensation is not necessary. The dosage of x-rays used could not have been at fault, for it invariably produced the glandular tissue when both ovaries were treated. The normal cycles were maintained by the one normal ovary, the atrophic irradiated ovary being sterile and without influence. However the irradiated ovaries must have been responsible for the increase in size of the pituitaries, for they were uniformly heavier than those of comparable control animals.

#### SUMMARY

One ovary of a pair was irradiated in order to produce in it interstitial gland, to determine the effect of the presence of such glandular tissue on the normal ovarian structure and the oestrous cycle. Unexpectedly, the presence of a normal ovary prevented the formation of glandular tissue in the irradiated one. The irradiated ovaries became atrophic. The normal ovaries continued to function normally. Oestrous cycles and oestrous openings were regular and typical.

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# A MORPHOLOGICAL AND EXPERIMENTAL STUDY OF THE INTRANUCLEAR CRYSTALS IN THE HEPATIC CELLS OF THE DOG

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THREE PLATES (TWENTY-SEVEN FIGURES)

In a study of the finer structure of the liver of the dog in anaphylactic shock (Weatherford, '35), attention was attracted to the presence of crystals within the nuclei of some hepatic cells. Intranuclear crystals have been described often in plant cells ever since they were observed by Radlkofer (1858, 1859) in the toothwort, *Lathraea squamaria*; but reports of them in animal cells have appeared less frequently and in widely unrelated species. Because of this sporadic occurrence in animal cells and the differences of opinion in interpreting them, it was considered that a systematic study of intranuclear crystals in the dog might be of interest.

Marchand (1887), investigating the toxicity of chlorates, observed, in an old dog, prismatic intranuclear crystals in the cells of the convoluted tubules of the kidney. Two years later Grandis (1889) confirmed Marchand, besides finding crystals in the nuclei of hepatic cells of dogs. Grandis was unable, however, to find crystals in the frog, turtle, pigeon, young rat, rabbit (normal and dead from inanition), sheep, cow, young cat, pig and man. Browicz (1897, 1898, 1899, '01 and '02), Herring ('06), Herring and Simpson ('06), Brandts ('09), Fiessinger ('11), Berg ('29 and '34), Cowdry and Scott ('30), and Nicolau and Kopciowska ('36) also have described intranuclear crystals in the livers of dogs. Besides in the dog Berg ('34) observed crystals in the liver of a fox.

The crystals have been variously interpreted. Marchand (1887) thought first that they were probably of protein origin (globulin?); but in 1909 after the finding of erythrocytes in both the cytoplasm and nuclei of hepatic cells by Browicz (1897, 1899), Herring ('06), Herring and Simpson ('06), and Brandts ('09) he believed, with them, that the crystals were perhaps hemoglobinous. Fiessinger ('11) stated that they resembled hematin. From more systematic studies on the crystals Grandis (1889) suggested the possibility that they were derived from nuclein. Berg ('29) also, from extensive studies, pointed out the absence of iron and the differences in crystal habit between the intranuclear crystals and hemoglobin, and expressed the likelihood that they were largely allantoin, the principal end product of purine metabolism in the dog. Cowdry and Scott ('30) noted clearly the differences between the crystals and certain rounded or ovoid intranuclear inclusions observed in the livers of some dogs and indistinguishable from those produced by filtrable viruses. Nicolau and Kopciowska ('36) did not make such a clear distinction between the crystals and other intranuclear inclusions and ascribed their formation to the action of a saprophytic virus.

Intranuclear crystalloids have been described in the cells of the sympathetic ganglia of the hedgehog by von Lenhossék (1897), Prenant (1897), and Sjövall ('01). These crystalloids presented a rod-like or filamentous form and stained with iron hematoxylin. Roncoroni (1896) believed he saw crystalloids with a polar orientation in the nuclei of middle-sized pyramidal cells and in the cells of the spinal cord of man. Lugaro (1896) was unable to confirm Roncoroni and thought that the latter was dealing with either an artifact of the nuclear membrane or a peripheral accumulation of chromatin. Cajal ('03) described in the cerebellum of the rabbit certain inclusions which he termed the 'intranuclear crystalloid bastonecytes.' In the English translation of his textbook ('33) the bastonecytes were illustrated as long, slender, flexible filaments impregnated with reduced silver

nitrate. Cajal referred to the bastonocytes as "a species of nuclear nutritive reserve, constantly present in the granule cells of the cerebellum, where it is very fine in outline, and in almost all the small or medium neurones of the cerebrum." Cajal accepted Roncoroni's observation, besides those of von Lenhossék (1897) and Holmgren (1899) in which were described similar structures.

Von Kölliker (1889) mentioned intranuclear crystals in cells of the germinal vesicle of fishes. Alden B. Dawson ('33) described, in detail, intranuclear crystalloids in the superficial epidermal cells of the catfish, *Ameiurus nebulosus*, but he did not attempt to identify them.

Among the invertebrates, intranuclear crystals have been described by Frenzel (1882, 1886), Rengel (1896), Biedermann (1898), and Magshikowskaja ('29) in the intestinal epithelium of the larva of the mealworm, *Tenebrio molitor*. Observations revealed that the size and number of crystals fluctuated with the nutritive conditions, since they could be increased by feeding and depleted by starvation. Mingazzini (1889) saw intranuclear crystals in cells of similar type in the lamellicorn larva. Carnoy (1884) depicted a large diamond-shaped crystal in the nucleus of a cell from the salivary gland of the common 'water scorpion,' *Nepa cinerea*. Cuénot (1891), Leipoldt (1893), St. Hilaire (1897), and List (1897) observed crystals in the nuclei of amoebocytes of echinoderms. The prevailing opinion of the several workers observing intranuclear crystals in cells of invertebrates was that they were reserves of protein.

#### MATERIAL AND METHODS

Sixteen dogs of different ages and breeds (ten mixed breeds, one German shepherd, one wire-haired fox terrier, and four Dalmatians)<sup>1</sup> are used for material. Anaphylactic shock is produced in four dogs sensitized to egg white. Pieces of

<sup>1</sup> The pieces of liver from the four Dalmatian dogs are obtained, at operation, through the courtesy of Prof. Harry C. Trimble of the Department of Biological Chemistry, Harvard Medical School.



liver from these and from four non-anaphylactic dogs are removed under local anesthesia and immersed for fixation in Regaud's potassium dichromate-formaldehyde mixture for 3 days, followed by a 3% aqueous solution of potassium dichromate, which is changed on alternate days for 1 week. Sections cut from this material are stained by the Heidenhain iron-alum hematoxylin, Mallory phosphotungstic acid hematoxylin, and Volkonsky modification of the Altmann aniline-acid fuchsin methods. Hepatic tissue from the other eight dogs is removed under Nembutal anesthesia and fixed in Heidenhain's 'susa,' formaldehyde diluted 1:9 with distilled water, formaldehyde diluted 1:9 with absolute alcohol, Zenker-Helly, Rabl's picric acid-sublimate, sublimate-acetic acid, and 2% solution of osmium tetroxide. All tissue, except some fixed in aqueous formaldehyde from which frozen sections are made, is embedded in paraffin and sections cut  $5\mu$  thick. Alternate sections of some of the tissue fixed in formaldehyde-alcohol are incinerated in a Policard micro-incineration apparatus. Other sections are stained with hematoxylin and eosin, Heidenhain's 'azan,' Okajima's phosphomolybdic acid lake of alizarin counterstained with hematoxylin, Best's carmine, and Sudan III according to Romeis. Various tests are applied to sections of suitably fixed material as the Lepehne benzidine, Turnbull blue, Prussian blue, Millon's, biuret, Ciaccio's, and Gersh's uric acid, and others.

#### OBSERVATIONS

1. *Occurrence, number and size of crystals.* Intranuclear crystals are found in variable numbers in the hepatic cells of dogs. An average of 5000 nuclei is counted in serial sections from each of two blocks of tissue from the same liver—1000 nuclei in every tenth section. From counts of more than 150,000 nuclei in sixteen dogs, the percentages of those containing crystals vary between 0 and 2.13%. The average for all dogs is 1.03%. This average is somewhat low, because in the four Dalmatian dogs counts reveal that only 0.26% of 17,000 nuclei hold crystals. In livers of dogs, other than

the Dalmatian, the average of crystal-containing nuclei is 1.27%. In all livers a lack of regularity is observed in the distribution of nuclei enclosing crystals. A comparison of nuclei containing crystals in two blocks of tissue from the same liver shows a presence of 2.02% in a piece excised near the center of the organ and 0.61% in the one cut from just beneath the surface.

Crystals are found in livers of dogs from 9 months old to senility, except in one dog of 2 years. In some young dogs a lower percentage of nuclei contains crystals, while in others a higher percentage of crystals is found than in some older dogs. No deductions can be drawn as to the relationship between feeding and the number of crystals. Some dogs are taken without regard to feeding, others are fed in the late afternoon and sacrificed the following day—18 to 20 hours intervening. Whether the number of intranuclear crystals is reduced by hunger or depleted by inanition no datum is available. The size of dogs, however, appears to be a factor; livers of larger dogs, except the Dalmatian, generally contain more crystals than those of smaller breeds.

Anaphylactic shock with its attending circulatory disturbances, passive hyperemia, perivascular infiltration, and passing of whole and disintegrating erythrocytes into hepatic cells, seemingly causes no change in the number of intranuclear crystals. Twenty-five thousand nuclei are counted in four dogs—two in mild shock and two in severe shock. The counts reveal that the percentages of nuclei with crystals, in each case, are within the same limits as in non-anaphylactic dogs.

The crystals lie in a clear area or vacuole within a nucleus. Usually only a single crystal is found. Sometimes, however, two or more crystals occur in the same or in separate clear areas or vacuoles. In binucleate cells usually only one nucleus contains a crystal, rarely both.

Generally crystals are seen in normally staining nuclei, but they occur also both in the pale and dark staining ones. They may be present, too, in the very large nuclei, which are observed concomitant with reparative processes. While

most plentiful in the nuclei of cells of normal size and staining, crystals are encountered in those of cells undergoing homogeneous atrophy. These cells, usually dark staining, are present frequently in the livers of dogs in anaphylactic shock. The nucleus and cytoplasm stain more or less uniformly, so in the later stages of atrophy the one seems to melt into the other. Crystals are seen emerging from nuclei, but in only one instance is detected an intact crystal in the cytoplasm of a hepatic cell. No crystals are found in the hepatic sinusoids. In one dog a medium-sized crystal is observed in a small tributary of a hepatic vein. This crystal is distinguished easily from the long needle-shaped hemoglobin crystals. Although no special search is made for crystals in dividing cells, once in the prophase, a crystal is seen lying between well-defined chromosomes.

Nuclei of a large number of tissues of the dog other than the liver reveal no crystals except in the proximal convoluted tubules of the kidney. Here the crystals lie in clear areas or vacuoles, their long axes parallel to the lumen of the tubule. Counts show that the percentages of nuclei containing crystals correspond, within limits, to those in the liver. Occasionally free crystals are seen in the lumen of a renal tubule.

Besides the crystals the nuclei of some hepatic cells present another inclusion. These latter inclusions are of various sizes, some small and rounded, others larger and ovoid or irregular. Like the intranuclear crystals they are surrounded by a clear area. One or more inclusions may occur in a nucleus. Occasionally they are observed in nuclei containing crystals and occupying the same or separate clear areas. Although no counts of inclusion bodies are made, they do not appear as numerous as the crystals nor are they found in all livers.

In nuclei containing crystals or inclusion bodies the chromatin is pushed excentrically and lies between the clear area or vacuole and the nuclear membrane. There is a tendency for chromatin to be condensed on the inner surface of the nuclear membrane rather than on the outer surface of the vacuoles surrounding crystals and inclusion bodies.

The shape of a nucleus depends upon the size and shape of the crystal it encloses. Very long crystals stretch the nuclear membrane until the nucleus in section is elliptical, while shorter crystals may or may not cause a change in nuclear shape. Two, three, or more crystals in a single vacuole frequently deform the nuclear shape, so it becomes irregular, ovoid, or elliptical in section. Multiple crystals in separate vacuoles, especially if they are large, likewise, deform the nucleus.

It is surprising how large a crystal can grow, and how much deformation of the shape of a nucleus can take place, without manifesting cytoplasmic alterations. A nucleus may become greatly elongated by a crystal without apparent alteration of chondriosomes other than their alignment parallel to its long axis. The shapes and staining of chondriosomes seem unaffected unless the cell is undergoing atrophy.

The crystals are present, mainly, as hexagonal prisms. Their cross sections are usually regular hexagons, but the hexagons may be lengthened with more or less suppression of two opposite sides. Rarely do the prisms have quadrilateral cross sections. Sometimes they are so short that they appear as hexagonal plates. Twinning is not infrequent. Bent crystals are encountered, besides those displaying signs of erosion. Sizes of the crystals are variable: some are very small, whereas others, usually slender, reach lengths of 25 to 35  $\mu$ . The average length is between 7 and 12  $\mu$ ; the maximum width is 5 to 7  $\mu$ .

Crystals are observed in scrapings from fresh liver. They are hard, transparent, pale yellow, and occupy a fluid-droplet in a nucleus. By microdissection they are isolated from nuclei. When a cell is entered, the nuclear membrane offers some resistance to the microneedle and is first indented before it is punctured. Then the fluid in the droplet is released carrying out the crystal. These isolated crystals, dried on a microscope slide, are studied with polarized light, filtered ultra-violet light, and chemically. Between the crossed Nicols



of the polarizing microscope the crystals appear highly refractive, but birefringence is not perceptible.<sup>2</sup> The dried crystals give no fluorescence. At room temperature crystals isolated from nuclei by microdissection do not dissolve in water, alcohol, ether, chloroform, benzol, xylol, or oil of cedar-wood. Dilute aqueous solutions of sodium and potassium hydroxides, lithium and sodium carbonates, and concentrated sulphuric acid cause them to swell and then dissolve. They are dissolved by concentrated nitric and acetic acids.

2. *Staining reactions and results of microincineration.* Intranuclear crystals in hepatic cells stain rose with eosin, red with 'azan,' pink with paracarmine, metachromatic blue with thionin, blue with phosphotungstic acid hematoxylin, and bluish-gray to black according to the length of staining and degree of differentiation, with iron-alum hematoxylin. Because of the staining with both acidic and basic dyes there is observed neither pronounced acidophilia nor basophilia.<sup>3</sup>

Sections of alcohol-formaldehyde (9:1) fixed livers are subjected to microincineration. For the earlier incinerations I am indebted to Dr. Gordon H. Scott of the Washington University of Saint Louis, School of Medicine. Doctor Scott reports:

There seems to be no iron ash in these crystals or if there is it is in such small amounts that it has escaped detection. Most of the ashed crystals that I observed gave a distinctly bluish residue quite separate from the remains of the chromatin minerals. This bluish appearance of the residue may indicate the presence of Na in the crystal or there is a possibility that it is due to the dispersion of the ash.

My own observations on incinerated preparations made later confirm Doctor Scott's report that the crystals seem iron free. Iron is detected in varying amounts in the cytoplasm of Kupffer cells and some hepatic cells. The color range is from a faint yellow to a brick red.

<sup>2</sup> The refractive index of the crystals is greater than that of dammar balsam, circa 1.520 (central illumination, Becke method and oblique illumination, Schroeder van der Kolk method).

<sup>3</sup> Zimmermann, 1893, intranuclear crystals in plant cells are erythrophil.



Microchemical tests for iron, the Turnbull blue and the Prussian blue, are applied to sections from the same livers, the sections previously having been treated with acidulated alcohol to 'unmask' the iron. The intranuclear crystals are negative to both of these tests, but blue granules are present in the cytoplasm of Kupffer cells and some hepatic cells.

3. *Experiments with hemoglobin.* The intranuclear crystals in the liver have been stated to be augmented some hours after intravenous injection of a hemoglobin solution. From a medium-sized female dog (wire-haired fox terrier) under Nembutal anesthesia a small piece of liver is removed, divided, one-half fixed in alcohol-formaldehyde solution and the other in Heidenhain's 'susa' mixture. Then the animal is given an intravenous injection of 25 cc. of a saturated aqueous solution of hemoglobin. Five hours later a second piece of liver is excised and treated like the first. The dog recovers quickly, and 2 weeks after the operation a third piece of liver is removed. Immediately following this the dog is given an intravenous injection of 250 cc. of the hemoglobin solution, a quantity sufficient to produce shock. After 5 hours a fourth piece of liver is taken and the dog killed.

Alternate sections from all tissue fixed in alcohol-formaldehyde solution are incinerated, and the ashed sections observed with dark-field illumination. No iron is detected in the ash of the intranuclear crystals. Increased amounts of iron are present both in the Kupffer cells and in the hepatic cells following the injections of hemoglobin.

To part of the remaining sections is applied the Lepehne benzidine test. Brown granules are shown in the Kupffer cells and hepatic cells, some even in the nuclei of the latter. Intranuclear crystals are negative. Okajima's phosphomolybdic acid lake of alizarin has been used for staining erythrocytes, although doubt has been cast upon its specificity for staining hemoglobin. The application of this stain, with hematoxylin for contrast, to sections of alcohol-formaldehyde fixed livers gives interesting but inconclusive results. Erythrocytes, certain granules in Kupffer cells and hepatic cells,

and, after injections of hemoglobin, needle-shaped crystals both in the cytoplasm and nuclei are stained a brilliant orange, while the intranuclear crystals show only a very light yellow. Besides these intracellular needle-shaped crystals, some Kupffer cells and hepatic cells contain whole erythrocytes in their cytoplasm, a picture comparable to the one seen in anaphylactic shock.

Sections from the blocks of liver fixed in Heidenhain's 'susa' mixture are stained with hematoxylin and eosin. In these sections a total of 40,000 nuclei are counted, 10,000 in each case—before injection of hemoglobin, 5 hours after injection of 25 cc., 2 weeks later, and 5 hours after injection of 250 cc. The results of the counts are surprisingly uniform, showing 12, 16, 17 and 19 crystal-containing nuclei in each instance. Although the figures chance to form a graded series, some counts of 1000 nuclei show no crystals whereas other 1000 nuclei exhibit as many as five. Consequently any differences are so slight as to be statistically meaningless.

Blood from the same dog is defibrinated by whipping, laked with ether, centrifuged, and put in a refrigerator over night. The result the next morning is a copious crop of crystals which are not readily soluble in cold, but dissolve easily in warm water. They occur mostly as elongated narrow prisms with a diamond-shaped cross section and terminate with a rather flat dome. The crystals display prominent longitudinal striations. Double refraction is marked.<sup>4</sup> The ratio of the length to thickness is very much greater than in the intranuclear crystals.

Because the intranuclear crystals are found devoid of iron, negative to the benzidine reaction, and of different crystallographic form than the hemoglobin crystals, it is considered that some derivative of hemoglobin should be sought for. The most probable derivative suspected is hemato-porphyrin.

<sup>4</sup> Reichert and Brown ('09) state that oxyhemoglobin in the dog displays two forms, one  $\alpha$ -oxyhemoglobin (orthorhombic) corresponding to observations recorded above, the others  $\beta$ -oxyhemoglobin (monoclinic) found sparingly and forming prisms and plates.

Frozen sections of fresh liver, also deparaffinated sections of liver fixed in alcohol-formaldehyde solution are examined with the fluorescence microscope. While most of the intranuclear crystals show no fluorescence, some fluoresce a pale bluish-white. Hepatic tissue gives a yellowish-green fluorescence. Hematoporphyrin is known to fluoresce red.

4. *Physical and chemical properties.* Intranuclear crystals in sections of alcohol-formaldehyde fixed livers do not stain with Best's carmine, while glycogen stores in the cytoplasm are colored bright red. Tincture of iodine stains the crystals a light yellow and the glycogen a deep reddish-brown. Sudan III does not stain the crystals in frozen sections of formaldehyde fixed livers or in scrapings from the fresh organ. Osmium tetroxide (2% aqueous solution) stains the crystals yellowish within 24 hours and brownish after 1 week. This staining by osmium tetroxide is interpreted as adsorption on the crystal faces.

Sections of formaldehyde fixed liver are tested for bile pigments by the method of Stein ('35). The sections from distilled water remain for 6 hours to overnight in Lugol's solution, then are washed in water and decolorized (15 to 30 seconds) in a 5% aqueous solution of sodium hyposulphite. After washing well in water, the sections are stained with paracarmine. Bile pigments should become emerald-green through the reaction. The intranuclear crystals show no green coloration, being stained only a faint pink by the paracarmine. Besides, the crystals withstand treatment without dissolving or apparent alteration with the usual histological reagents such as alcohol, ether, chloroform, benzol, xylol, et cetera. Bilirubin is slightly soluble in alcohol and soluble in chloroform and benzol. Biliverdin is soluble in alcohol and benzol, slightly soluble in ether, and insoluble in chloroform.

Because hemoglobin and its derivatives, carbohydrates, fats, and bile pigments are eliminated as possibilities for the intranuclear crystals, tests are made for proteins. Millon's reagent, which gives positive results for proteins containing

a hydroxy-phenyl group and certain non-proteins as tyrosine, is negative for the intranuclear crystals. The biuret reaction given by substances with two amino groups also is negative. Berg ('29) states that he obtained weakly positive results with the ninhydrin reaction. "This test gives positive results with proteins, peptones, peptides, and amino acids which possess a free carboxyl and  $\alpha$ -amino group" (Hawk and Bergeim, '26).

The consistent low counts of nuclei with crystals in livers of Dalmatian dogs cause a purine base to be surmised. All of the five purine bases 1) adenine, 2) guanine, 3) hypoxanthine, 4) xanthine, and 5) uric acid are precipitated by cold ammoniacal silver nitrate solutions. The intranuclear crystals are removed from sections by the ammoniacal silver nitrate in Ciaccio's and in Gersh's methods for detection of uric acid. All but the largest crystals are removed by the short treatment. Crystals or their remnants, where still present, are brownish-black. Counts made on sections, following the application of Gersh's method, reveal that only 0.3% of the nuclei contain crystals, whereas in untreated sections from the same block 0.61% of the nuclei have crystals.

Purine bases are precipitated from solution by copper sulphate and sodium bisulphite at the boiling point. When these reagents are applied to sections, as in Saint-Hilaire's method (Lison, '36), a number of nuclei show empty spaces, whereas others contain eroded crystals. In a nucleus containing two crystals in separate vacuoles, one 12  $\mu$  long is badly eroded the whole center being dissolved out and the edges ragged, while the other 16  $\mu$  long shows some wearing away.

Intranuclear crystals are removed, in part, from sections (cut from the same block of alcohol-formaldehyde fixed liver) immersed for varying periods in different solutions.

After heating 10 minutes in 5% aqueous solutions of barium chloride and calcium chloride no perfectly intact crystals are encountered in sections. Only a few badly eroded ones remain. Clear areas or vacuoles once holding crystals are



empty or contain some pale bluish punctate granules (H. and E. stain), presumably precipitations from the disintegration of the crystals.

Dilute sulphuric acid has little or no effect on the crystals. More concentrated acid (60 to 80%), while disastrous to sections, is effective in isolating crystals of similar habit from dried and powdered liver. Picric acid in fixation solutions,

TABLE 1

*Tabulation of the number of nuclei containing crystals in untreated and treated sections*

| TREATMENT                   | TIME       | NUCLEI<br>COUNTED | NUCLEI<br>WITH<br>CRYSTALS | PER CENT<br>CRYSTALS |
|-----------------------------|------------|-------------------|----------------------------|----------------------|
| Untreated—dog B             |            | 7000              | 43                         | 0.61                 |
| Sodium hydroxide N/50       | 15 minutes | 1000              | 5                          | 0.50                 |
| Sodium hydroxide N/50       | 30 minutes | 1000              | 3                          | 0.30                 |
| Sodium hydroxide N/50       | 1 hour     | 1000              | 1                          | 0.10                 |
| Ammonium hydroxide 1:2      | 1 hour     | 1000              | 3                          | 0.30                 |
| Ammonium hydroxide 1:2      | 3 hours    | 1000              | 2                          | 0.20                 |
| Lithium carbonate 5% aq.    | 24 hours   | 2000              | 8                          | 0.40                 |
| Nitric acid 10%             | 24 hours   | 2000              | 12                         | 0.60                 |
| Nitric acid concentrated    | 1 hour     | 2000              | 3                          | 0.15                 |
| Ferric chloride 5% aq.      | 1 hour     | 2000              | 7                          | 0.35                 |
| Phosphotungstic acid 5% aq. | 3 hours    | 2000              | 7                          | 0.35                 |
| Chlorine water              | 24 hours   | 2000              | 6                          | 0.30                 |
| Urea 6% aq.                 | 24 hours   | 2000              | 8                          | 0.40                 |
| Boiling water               | 10 minutes | 2000              | 8                          | 0.40                 |
| Boiling water               | 1 hour     | 2000              | 5                          | 0.25                 |
| Untreated—dog 20            |            | 2000              | 15                         | 0.75                 |
| Piperazine 5% aq.           | 15 minutes | 1000              | 6                          | 0.60                 |
| Piperazine 5% aq.           | 30 minutes | 1000              | 5                          | 0.50                 |
| Piperazine 5% aq.           | 1 hour     | 1000              | 3                          | 0.30                 |

besides in staining, does not remove the crystals from sections but colors them yellow. Phosphotungstic acid in Mallory's hematoxylin and in Heidenhain's 'azan' method appears to have little or no effect on the number of crystals in the nuclei. Sections stained by both of these methods show no variation as to crystal content from those stained with hematoxylin and eosin. Perhaps the adsorption of the dye on the crystal faces has protected them from the dissolvent action of the phosphotungstic acid.



Glycerol dissolves the crystals very slowly, even 1 week to 10 days immersion does not remove all of them from sections. Hot glycerol, on the contrary, is a fairly efficient solvent for the crystals.

Intranuclear crystals are not removed from sections after 24 hours, at room temperature, by solutions of formaldehyde (40, 10 and 4%); but on heating, 40% formaldehyde dissolves them rather easily. After staining and dehydrating the sections, no small crystals are found. Medium sized and large crystals are either partly removed or show the effects of much erosion. In one nucleus very much elongated by a large crystal, the crystal was dissolved away to a mere strand in an elliptical vacuole. Hexagonal cross sections of crystals display jagged edges and worn-off corners. An unanswerable question arises—is the effect on the crystals caused by the formaldehyde or the hot water? Uric acid is said to dissolve easily in 40% formaldehyde and in other aldehydes on heating.

Crystals are no longer found in sections from pieces of liver kept in sterile containers for 3 days at 37°C. Moreover they are not found in liver incubated for the same length of time in a saturated aqueous solution of uric acid.

An attempt is made to see whether or not intranuclear crystals can be isolated by chemical means from dog's liver in sufficient quantity for analysis. A 16-kg. dog is anesthetized with Nembutal (0.65 cc. veterinary, per kilogram body weight). The liver is removed, two small pieces fixed for histological study, and the rest cut into small pieces. After washing in iced water to remove the surplus blood, 300 gm. of the liver are covered with about 3 volumes of 95% alcohol. The mass is stirred frequently until reduced to a pulp and then allowed to macerate for 24 hours. After filtering the pulp is dried under an electric fan, ground in a mortar, and passed through a fine sieve to separate the parenchyma from the connective tissue. The resulting powder is triturated with ground glass, covered in a closed jar with chloroform and allowed to stand 24 hours, then filtered and the

powder dried. Because the glass is heavier than the powdered hepatic parenchyma the two are separated easily. The powdered liver is passed through sieves of increasing fineness and forms the stock powder used in the following extractions.

Separate portions of the powder are extracted with hot 80% sulphuric acid, 10% nitric acid, N/10 sodium hydroxide, 5% lithium carbonate, glycerol, and cold pyridine. After appropriate treatment,<sup>5</sup> in each case, the liquid is concentrated and the crystalline product allowed to precipitate. Examination with the polarizing microscope reveals in each of these extractions birefringent prisms and plates identified as belonging in the orthorhombic system.

From the sulphuric acid extraction are obtained numerous and large crystals—from the pyridine and glycerol extractions the crystals are smaller, both in number and size. Besides the prisms, nitric acid extracts a large number of platy crystals belonging in the hexagonal system. Sodium hydroxide and lithium carbonate, like hot sulphuric acid, are quite efficient in extracting crystals from dried and powdered liver. Crystals from each extraction are similar in crystallographic habit and solubility in the same reagents as the intranuclear crystals; although the former show double refraction, the latter display no birefringence. It must be emphasized, however, that it cannot be stated whether the extracted crystals come from nuclei or not.

An extract of powdered liver prepared with boiling water and filtered, when cold, deposits a dense precipitate on adding an ammoniacal silver nitrate solution. From some of the extract, first made alkaline with sodium hydroxide, then faintly acid with acetic acid, and brought to boiling, a yellow precipitate forms on adding a 10% aqueous solution of copper sulphate and a few drops at a time of a saturated aqueous solution of sodium bisulphite.

When the final liquids from each of the above extractions of powdered liver are evaporated on a water bath, moistened

<sup>5</sup> Levene and Bass ('31).

with concentrated nitric acid, and heated to dryness, they develop a yellow color; on adding a drop or two of dilute ammonium hydroxide the color changes to a reddish-purple—the murexide reaction.

An aqueous solution of each of the crystalline products of the described extractions responds to Folin's phosphotungstic acid reagent by developing a blue color. The depth of color is compared with that developed by a standard solution of uric acid treated in like manner. The color depths are in proportion to the efficacy of the various methods of extraction.

#### DISCUSSION

The observations indicate a variability in the number and distribution of hepatic cell nuclei containing crystals. All authors attest to this but hitherto only Berg ('29) has recorded actual counts of them. Extensive counts of nuclei (over 150,000) confirm the percentages obtained by Berg.

Crystals are found in fifteen dogs from 9 months old to senility, the only exception being one dog of 2 years. Marchand (1887) observed intranuclear crystals in renal cells of an old dog. Grandis (1889) saw crystals in varying quantity in fifteen of eighteen adult dogs but could not find them in eight young dogs. Ages were not given. Brandts ('09) found no crystals in young dogs (6 months to 3½ years) but observed them in older dogs (6 to 8½ years). Dogs between these ages were not studied. Berg ('29) found crystals in twenty-one dogs from 9 months to 20 years old. Nicolau and Kopciowska ('36) saw hepatic intranuclear inclusions in twenty-seven of forty-four dogs, twenty-six over 2 years old and one of 11 months. In the kidneys of the same twenty-seven dogs, ten over and two less than 2 years old had inclusions. Age was considered important but since Nicolau and Kopciowska did not distinguish clearly between the crystals and other intranuclear inclusions, no deductions can be drawn from their findings. Perhaps age is important, although my counts show that some young dogs have a larger percentage of nuclei with crystals than other older dogs.

Nicolau and Kopciowska stated that the breed of dog played no role. While no distinction can be made as to the relationship between breed and crystal content, except in the Dalmatian, larger dogs generally have more crystals than smaller ones.

The presence of crystals, whatever their nature, must be caused by some peculiarity in the metabolism of the dog, since crystals are found only in the liver and kidney and have not been seen in the same organs of other common laboratory animals. It is surmised therefore that an altered metabolism would influence the number of crystals. While feeding experiments are not conducted, short fasts seemingly do not affect the number of nuclei with crystals, since they are plentiful in dogs 18 to 20 hours without food and in Brandts' ('09) animals killed about 30 hours after feeding. Grandis (1889) stated that crystals were absent after 3 to 5 days fast. There is no indication, however, from his study that pieces of liver were taken from the same dog before and after fasting. He found also no crystals in liver after 3 days autolysis, which I confirm from tests on pieces of liver known to contain crystals before autolysis set in.

The earlier observers (1887 to '09), Marchand, Grandis, Browicz, Herring, Herring and Simpson, and Brandts described the crystals as prisms with quadrilateral cross sections, although Brandts ('09, taf. XXV, fig. 14) illustrated a hexagonal crystal. Berg ('29) described them as prisms with a hexagonal cross section, while Cowdry and Scott ('30) stated that they were hexahedra and pentahedra. Observations show that while most crystals are presented as hexagonal prisms, by cleavage two of the opposite faces may be suppressed until on occasion the bases appear quadrilateral. The failure of the several earlier observers to see the hexagonal shapes is perhaps because of viewing the faces of the transparent crystals and not the bases.

There seems no doubt in the minds of all investigators on the problem, except Browicz, that the intranuclear crystals are present in living cells. Browicz (1897) considered a



possible post-mortem origin brought about by fixation. He did not mention a study of living hepatic tissue and either he was unaware of or did not consider Grandis' findings on scrapings from fresh liver. Herring ('06) wrote:

The crystals must be formed during the life of the liver cell; the cell is presumably killed by the fixative employed and if the crystals are of post-mortem formation the nuclear membrane must inevitably be ruptured and the cell protoplasm torn. Nothing of the kind occurs and both nucleus and cell have the appearance of having completely adapted themselves to the presence of the crystal during life.

This adaptation of the nucleus to the enclosed crystal seems very evident, because the majority of crystals are found in cells without observable alterations. Moreover, when the lability of the hepatic cell is considered and the susceptibility of chondriosomes to injury is taken into account, it appears incontrovertible that the crystals are present in living cells. Furthermore, my observations, in agreement with Grandis (1889), Brandts ('09), and Berg ('29) on crystals in fresh liver scrapings and with Berg on the isolation of crystals by microdissection, indicate that they are not caused by fixation.

From the studies with microdissection the crystals are seen within fluid-droplets, and it is surmised with Berg ('34) that these contain the mother liquor for the crystals, and further that crystallization takes place when this fluid becomes concentrated enough. Perhaps insufficient concentration accounts for the failure to find crystals in the nuclei of other animals than the dog.

Browicz (1897 to '02) observed erythrocytes in hepatic cells under certain pathological and physiological conditions, also after intravenous injections of hemoglobin and transfusions of foreign defibrinated bloods. Because of the similarity in the staining of the erythrocytes, hemoglobin, and intranuclear crystals, he concluded that the latter were hemoglobinous in origin. It was postulated that hemoglobin in solution and from disintegrating blood cells crystallized out



in needles and, becoming modified in the nucleus, was subsequently recrystallized by fixation—possibly as methemoglobin. The sizes of the larger intranuclear crystals were stated to correspond with the finding of more erythrocytes in hepatic cells. Herring ('06), Brandts ('09), Marchand ('09), and Fiessinger ('11) accepted, in essence, the hemoglobinous origin for the intranuclear crystals.

In anaphylactic shock, where there is considerable blood destruction and appearance of erythrocytes in hepatic cells, I find no larger number of intranuclear crystals than in dogs used as controls. A repetition of Browicz's experiment on the injection of hemoglobin, but with pieces of liver removed both before and 5 hours after injection, shows that the crystals are neither larger nor more numerous after the injection. Herring ('06) stated that it was unnecessary to inject either hemoglobin or hemolytic substances to demonstrate the crystals and Brandts ('09) found no crystals in the livers of two young dogs—6 and 8 months old—4 hours after injection. Moreover, hemoglobin crystallized from blood taken before the injection is dissimilar crystallographically to the intranuclear crystals. Further, microincinerations reveal no iron in the intranuclear crystals, confirming both Grandis (1889) and Berg ('29) who found iron absent in the ash of isolated crystals.

The consistent low counts of nuclei with crystals in the livers of four pure bred Dalmatian dogs causes a purine base to be suspected. The principal end product of purine metabolism in the dog is allantoin, but Benedict ('16) found the Dalmatian to be an exception in having a high uric acid and a low allantoin elimination. Uric acid injected into the Dalmatian dog was eliminated as such, whereas 98 to 100% of the uric acid injected into other breeds of dogs was destroyed. Prof. Harry C. Trimble tells me that his pure bred Dalmatians eliminate from five to seven times as much uric acid a day as other dogs.

Bollman, Mann and Magath ('25) showed that destruction of uric acid and its conversion into allantoin in the dog depended upon the liver, because after hepatectomy there was

an increased excretion of uric acid. They stated that the uric acid excreted by a hepatectomized dog was of the same magnitude as the allantoin excreted by a normal dog.

It has been generally taught that the presence of an enzyme—uricase—in the liver, spleen and perhaps other organs of dogs rapidly destroys uric acid so that it appears in the urine as allantoin. The absence of this enzyme in the tissues of man and perhaps in the chimpanzee and the Dalmatian dog leads to excretion of uric acid, but an absence of uricase has been controverted.

Folin, Berglund and Derick ('24) stated that uric acid injected into the blood stream (dogs, cats, rabbits, goats) was absorbed immediately by the kidneys and up to 0.2% might be stored there temporarily. Other tissues were regarded as more or less completely impermeable to soluble urates. Destruction of uric acid took place in the blood and possibly in the kidneys; in the circulating blood it was destroyed rapidly. After the blood was removed from the living animal uric acid destruction stopped. Folin et al. believed that some unknown essential factor must be contributed by the tissues, possibly the liver, and that this unknown oxidizing agent was apparently used up as rapidly as it was poured into the blood, and therefore could scarcely be an enzyme.

Wells ('18) found uricase present in the liver of the Dalmatian dog, indicating the excretion of uric acid was not caused by the absence of uricolytic enzymes. For additional data see Hunter and Ward ('19) and Rose ('23).

Purine bases—and allantoin (Wiechowski, '13)—are precipitated from solution by cold ammoniacal silver nitrate. Intracellular crystals are removed, in part, by cold ammoniacal silver nitrate solution and reduced silver is deposited on the surfaces of the remaining ones and their remnants. Furthermore, the intracellular crystals and those of uric acid become brown within 10 minutes when put into a 10% aqueous solution of silver nitrate, whereas crystals of allantoin remain uncolored.

Ciaccio ('08) demonstrated purine bodies in the liver of a cat with ammoniacal silver nitrate solution and found them augmented during the digestion of meat, after intraperitoneal injections of nucleic acid, and in the early stages of infections and intoxications. While the feeding of meat may increase the exogenous purines and infections and intoxications may free endogenous ones—adenine, guanine and hypoxanthine—from nucleic acid, some condition must be present in the dog causing a crystallization and storage in hepatic and renal nuclei which is not found in the cat.

Melzer ('26) was skeptical of the silver impregnation methods for uric acid, because he could find always with the polarizing microscope more uric acid in unimpregnated sections than in silvered ones. He tried the effects of osmium tetroxide in concentrations of 0.25 to 2% on pure uric acid, urates and tissue. Easily soluble urates were not influenced but uric acid and the insoluble lead and calcium urates were blackened after 64 hours by higher concentrations (1 to 2%) of osmium tetroxide.

Two per cent osmium tetroxide colors the intranuclear crystals yellowish within 24 hours and brownish after 1 week. Grandis (1889) made similar observations and Frenzel (1886) and Mingazzini (1889) in the intestinal epithelium of insect larvae found crystals browned by osmium tetroxide. List (1897) saw six-sided and rhomboid crystals in the nuclei of amoebocytes of echinoderms which became brownish after fixation in Flemming's fluid.

A comparison of the solubilities of the intranuclear crystals with those known for purine bases and allantoin shows similarities—insoluble or very slightly soluble in water, alcohol, ether and chloroform; easily soluble in solutions of alkali hydroxides and soluble with difficulty in ammonium hydroxide; and irregularly soluble in mineral acids. The intranuclear crystals are seemingly unaffected by 10% sulphuric acid but swell and disappear in concentrations of more than 60%. Dilute (10%) nitric acid removes crystals slowly from sections while concentrated acid is more rapid in removing them

and dissolving those isolated by microdissection. Only part of the crystals are removed from sections in 24 hours by N/10 hydrochloric acid.

Grandis (1889) found isolated crystals dissolved in 10% mineral acids. Berg ('29) stated that they dissolved in  $1\frac{1}{2}$  to 2 N hydrochloric and sulphuric acids. Brandts ('09) observed the crystals swelling and then disappearing in concentrated sulphuric acid.

Phosphotungstic acid removes intranuclear crystals from sections. Purine bases are precipitated from solution by phosphotungstic acid while allantoin is not (Wiechowski, '13). The addition of Folin's phosphotungstic acid reagent for uric acid to solutions from the various extractions of powdered liver causes a blue color to develop; but since these solutions contain a mixture of crystals, including some of similar habit to the intranuclear crystals, no deductions are drawn other than that the extractions contain uric acid.

Isolated intranuclear crystals are dissolved by acetic acid—similar findings were reported by Grandis (1889). Guanine is insoluble and hypoxanthine is precipitated by acetic acid (Levene and Bass, '31).

Mixtures of crystals isolated from powdered liver show a positive murexide reaction. While generally used for detection of uric acid a positive reaction is given by guanine (Levene and Bass, '31), xanthine and its methyl derivatives (Lison, '36), but allantoin does not respond (Wiechowski, '13).

Picric acid colors the intranuclear crystals yellow but does not remove them from sections. Both adenine and guanine are precipitated by picric acid and adenine is separated from hypoxanthine by its almost insoluble picrate. Tincture of iodine (U.S.P.) colors intranuclear crystals yellowish. Iodine-potassium iodide solutions precipitate guanine and xanthine. Cold glycerol removes intranuclear crystals from sections very slowly, while hot glycerol is more rapid. Uric acid dissolves in hot glycerol. Intranuclear crystals are removed from sections by solutions of piperazine which also dissolve



uric acid and allantoin. According to Wiechowski ('13), Salkowski found allantoin more soluble in piperazine than in water. Guanine is insoluble in piperazine (Lison, '36).

Berg ('29) considered the intranuclear crystals as probably largely allantoin, since it is the principal end product of purine metabolism in the dog and also, because of the six-sided prismatic crystals. The crystals were stated to belong in the hexagonal system. The crystallographic habit of allantoin is well known: the International Critical Tables and the various handbooks place it in the monoclinic system. Studies on three samples of allantoin, two from commercial sources and one prepared personally by the oxidation of uric acid with potassium permanganate show monoclinic crystals. I am inclined to place the intranuclear crystals in the orthorhombic system, despite the difficulties encountered in making full studies on them.

Grandis (1889), Berg ('29), and the findings in this study point out that the intranuclear crystals exhibit no double refraction. Uric acid, urates, and allantoin crystals are birefringent. Ordinarily this would exclude them as possibilities for the intranuclear crystals, but when contaminations and surface adsorption are taken into account, double refraction may be rendered imperceptible. Thinness of the intranuclear crystals is not a factor, because selected crystals of both uric acid and allantoin are embedded in paraffin and sections cut at the same thickness as liver and they still show double refraction.

Additional tests with ultra-violet light indicate that the crystals are not allantoin. Dry uric acid gives no fluorescence, while in aqueous solution it fluoresces a pale bluish-white—the color increasing slightly on adding acetic acid. Allantoin and its aqueous solution fluoresce deep blue. Intranuclear crystals either give no fluorescence or show at the most a slight bluish-white. Absence of fluorescence by uric acid was recorded by Radley and Grant ('33) and Danckwortt ('34).

It is concluded from the physical and chemical studies on the intranuclear crystals that they are derived from a purine base although the exact compound remains still unidentified.



## SUMMARY

1. Intranuclear crystals are present in varying numbers in the hepatic cells of the dog—up to 2.13% of the nuclei containing them. In the livers of sixteen dogs, 9 months old to senility, an average of 1.03% of the nuclei include crystals. The Dalmatian dog is exceptional in having consistently fewer crystals; the average count for the four dogs is 0.26%.

2. The crystals occur as hexagonal prisms, usually single but sometimes multiple in a nucleus. In binucleate cells rarely do both nuclei contain crystals. Their lengths range from very short prisms up to 25 to 35  $\mu$ . The average length is between 7 and 12  $\mu$ ; the maximum width is 5 to 7  $\mu$ . In polarized light the crystals are highly refractive but no double refraction is seen. Usually no fluorescence is shown in ultra-violet light.

3. Hepatic cells seem to adapt themselves to the presence of crystals and neither the chondriosomes nor the storage of glycogen appear altered by them. Short fasts, anaphylactic shock, and intravenous injections of hemoglobin do not change the number of crystals in the nuclei.

4. Crystals isolated from fresh liver by microdissection are insoluble in water, alcohol, ether, chloroform, benzol, xylol and oil of cedarwood. Dilute solutions of sodium and potassium hydroxides, and sodium and lithium carbonates cause them to swell and then dissolve. Concentrated sulphuric, nitric and acetic acids dissolve them.

5. In staining, the crystals exhibit neither a pronounced acidophilia nor basophilia. They are colored various shades of yellow by tincture of iodine, picric acid, and osmium tetroxide—the latter turns them brown within 1 week. Crystals are fat free (Sudan III) and iron free (Turnbull blue and Prussian blue reactions and microincineration).

6. Cold ammoniacal silver nitrate solution and boiling solution of copper sulphate and sodium bisulphite, which precipitate purine bodies, remove part of the crystals from sections. Also all of the following partially remove crystals: sodium and ammonium hydroxides, lithium carbonate, nitric acid (10%

and concentrated), ferric chloride, phosphotungstic acid, chlorine water, aqueous solutions of urea and piperazine, boiling water and glycerol.

7. It is concluded from physical and chemical studies that the crystals are derived from a purine base although the exact compound remains unidentified.

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## PLATE 1

## EXPLANATION OF FIGURES

1 to 5 Intranuclear crystals in hepatic cells of the dog. The rounded nucleoli are as intensely stained as the prismatic crystals, the latter in vacuoles. Chondriosomes apparently are uninfluenced by crystals in nuclei.

1 Three cells with intranuclear crystals—mid-zone of the lobule.

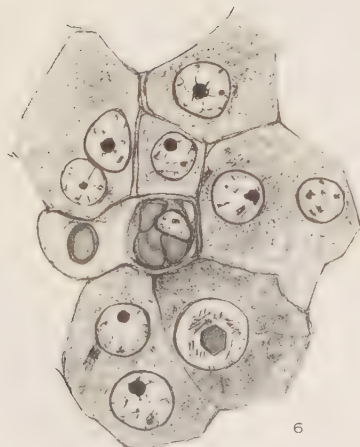
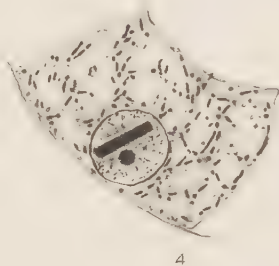
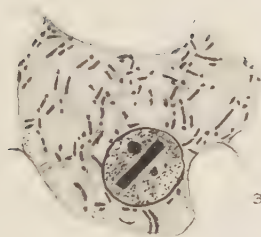
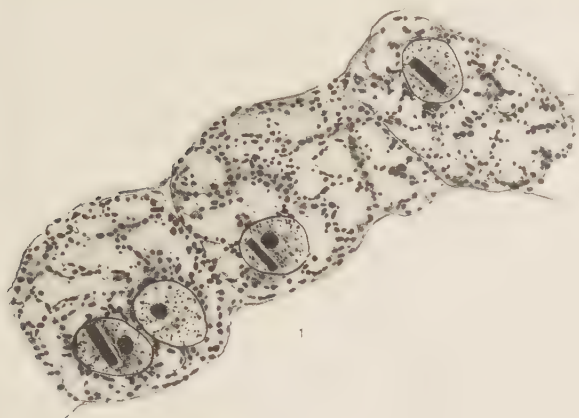
2 to 4 Three cells from the peripheral zone of the lobule, one a binucleate cell with crystals in both nuclei.

5 Group of three dark cells at the periphery of a lobule, one containing an intranuclear crystal. Notice the abundance of rod-shaped and filamentous chondriosomes. Regaud fixation: Mallory's phosphotungstic acid hematoxylin stain.

6 Five hours after intravenous injection of hemoglobin needle-shaped crystals are found in cytoplasm and nuclei of hepatic cells. One cell shows a hexagonal cross section of an intranuclear crystal. Heidenhain's 'susa' fixation: hematoxylin and eosin stain.

Zeiss apochromatic oil im. obj., 1.5 mm., N.A. 1.3 and 5× comp. oc. Camera lucida outlines. Figures 1 to 5, × 1200; figure 6, × 1000.





E. R. M.

## PLATE 2

### EXPLANATION OF FIGURES

7 to 21 Drawings of nuclei and contained crystals showing prismatic faces and hexagonal cross sections of crystals.

10 Bent crystal.

12 Transverse fracture of a crystal.

13 Two prisms in a single vacuole.

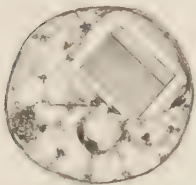
14 Two prisms in separate vacuoles.

15 Cross sections of two crystals in separate vacuoles. The larger crystal is evidently atypical because opposite faces are not parallel.

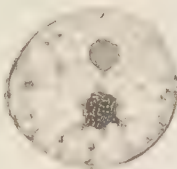
16 Multiple crystals, cross sections and faces in the same vacuole. Compare with figure 21, cross sections of four crystals in separate vacuoles.

19 Cross section, showing effects of cleavage. Rabl's picric acid-sublimate and Zenker-Helly fixations: hematoxylin and eosin stain.

Zeiss apochromatic oil im. obj., 1.5 mm., N.A. 1.3 and 15 $\times$  comp. oc. Camera lucida outlines.  $\times$  3000.



7



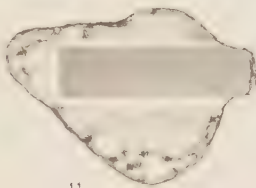
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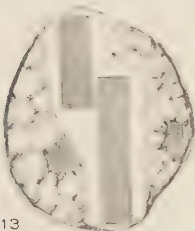
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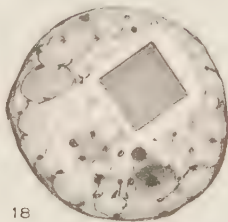
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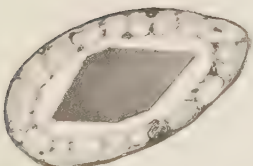
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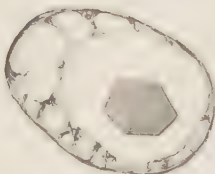
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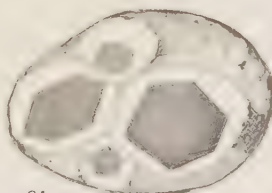
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*E. Roth*

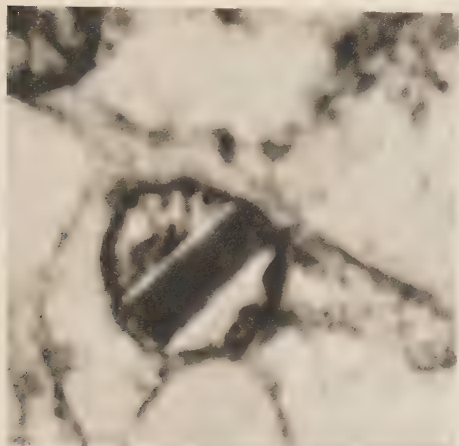
### PLATE 3

#### EXPLANATION OF FIGURES

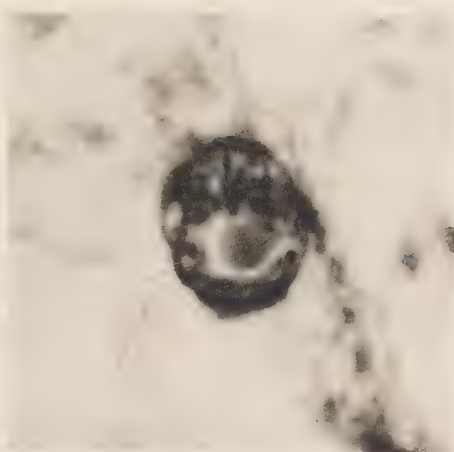
22 to 27 Unretouched photographs of crystals in nuclei.

22 to 26 Zeiss apochromatic oil im. obj., 2 mm., N.A. 1.4 and  $15\times$  oe.  $\times 3000$ .

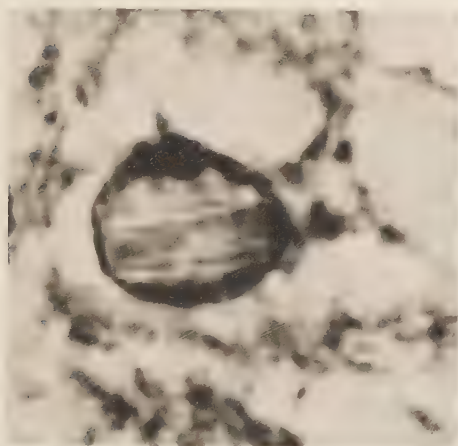
27 Zeiss apochromatic dry obj., 4 mm., N.A. 0.95 and  $10\times$  oe.  $\times 1500$ .



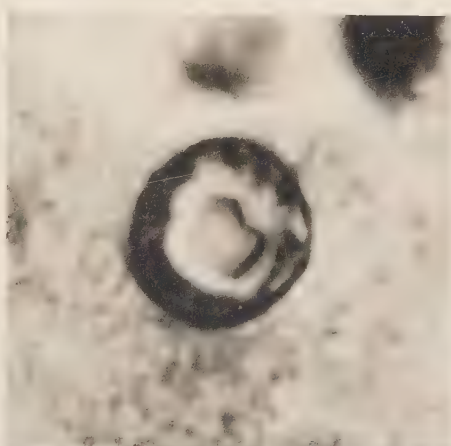
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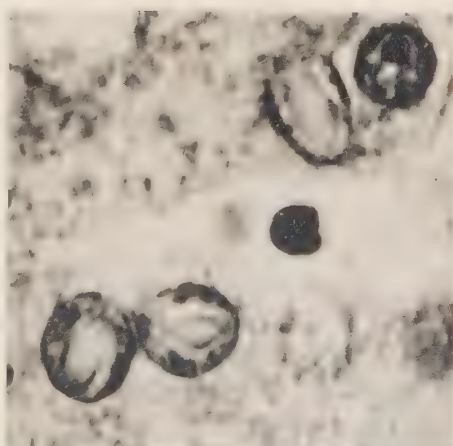
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# THE EFFECTS OF CRYSTALLINE SEX HORMONES ON SEX DIFFERENTIATION IN AMBLYSTOMA

I. ESTRONE

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THREE PLATES (TWELVE FIGURES)

## INTRODUCTION

The preparation and synthesis in recent years of a variety of chemically pure crystalline substances, having the properties of the naturally occurring sex hormones, has provided a more direct method of attack on the problems of sex differentiation than has been available hitherto. The administration of exact dosages of standard preparations is replacing the use of cruder extracts standardized in units which have different values according to the manner of preparation, the form investigated, and the particular structure used as a test object. Since these substances (and their various compounds) represent sex hormones in the forms in which they have been recovered from the tissues or body fluids of mammals, it is naturally of interest to try out their specific effects and relative potencies on the various parts of the developing reproductive systems of lower vertebrates.

This program has already been undertaken extensively in the incubating chick. The first experiments of this type were carried out by Kozelka and Gallagher ('34), but subsequently the most complete analyses of the field and the problems raised are those of Willier, Gallagher and Koch ('35, '37), Wolff and Ginglinger ('37), and Willier ('37). The findings with respect to several hormones, indicate on the whole a specific action

on structures of the appropriate sex, with exceptions in the case of the male hormone androsterone and its derivatives, which appear to manifest a dual activity. (Wolff and Wolff, '37, also report that testosterone acetate exerts a double action.) Many interesting problems arise, especially with reference to the mode of action of the sex hormones during development; and although the evidence is frequently suggestive, many points are not as yet finally established.<sup>1</sup>

Similar results have been obtained by Forbes ('38) in the young alligator, and by Risley ('37) in a preliminary note on the turtle embryo. There is, however, the paradoxical result of Padoa ('36) who found that a female hormone of follicular origin, administered to young tadpoles of *Rana esculenta*, induced the differentiation of male gonads only; a result further complicated by the fact that the same type of hormone, administered to trout fry (Padoa, '37), had an opposite and orthodox effect, producing various degrees of histological reversal in differentiating testes. Ovaries were not affected except with high concentrations, when there was sometimes an inhibitory action without the paradoxical effect found in the frog. On the other hand there is the preliminary statement of Witschi and Crown ('37) who failed to obtain Padoa's 'paradoxical effect' in tadpoles of *Rana pipiens* when the female hormone 'dihydrotheelin' was used. Consequently there is much to be done before we shall arrive at a common basis for resolving these inconsistencies; if indeed a common basis exists in a strict sense for all vertebrates. The new crystalline hormones and their chemical allies should therefore be tried out widely in the lower classes of vertebrates with a view to correlating their effects on the various parts of the sex equipment at different stages of development.

<sup>1</sup> This work has been reviewed recently by Willier in the forthcoming edition of *Sex and Internal Secretions* (Williams and Wilkins, Baltimore, 1938) and by Burns ('38).

## THE EXPERIMENT

This report deals with the administration of crystalline estrone<sup>2</sup> by injection over prolonged periods of time, to young larvae of *Amblystoma punctatum* (Linn.). The hormone dissolved in sesame oil was administered by injection into the body cavity. Due to the small size and delicacy of the organisms at the beginning of the experiment, a modified micro-injector was used, capable of delivering a small volume (as low as 0.001 cc.) accurately, with the subjects under light anesthesia. This apparatus proved to be suitable for work well beyond the possibilities of a hand syringe, without the complications of more refined micro-injectors requiring mechanical manipulation. Injections were begun when histological examination of controls showed that sex differentiation was under way. Earlier injection would have been possible, but was not attempted on account of the small size of the subjects and lack of acquaintance with the possibilities of the injection apparatus at the beginning. Previous experience in the administration of hormones to amphibians had shown that higher concentrations are necessary and a longer period of action is required, as compared with warm-blooded animals; while frequent injections of small volumes are more satisfactory than larger volumes at longer intervals.

The experimental subjects were twenty-five larvae of *A. punctatum* from the Ozark region of Arkansas, of the same age and carefully selected for size. All had been reared in the laboratory for some time at a slow rate of growth, a function easily regulated within considerable limits by controlling the temperature and restricting the food intake. Due to the slow rate at which development had proceeded they were only 35 mm. in length at approximately 75 days of age, when the experiment was begun. Individuals received twelve to fifteen injections of 0.005 cc. of the hormone solution at 3- to 4-day intervals over a period of 50 to 60 days, an injection containing 10  $\gamma$  of the crystalline substance. Three died

<sup>2</sup> For the hormone used we are indebted to the Schering Corporation and especially to the kindness and interest of Dr. Erwin Schwenk.

within the first week, apparently because of injury while injecting, and were discarded. Ten were preserved for study at the end of 50 days (twelve injections), and the remaining twelve were sacrificed 10 days later, after receiving a total of 150  $\gamma$ , when unusually warm weather threatened the colony. The injections were evenly distributed, with one interruption of 7 days when most of the animals were in the midst of

TABLE 1  
*Estrone administration*

| DATE OF INJECTION         | AGE         | LENGTH     | DOSE          | NUMBER OF CASES | COMMENT            |
|---------------------------|-------------|------------|---------------|-----------------|--------------------|
|                           | <i>days</i> | <i>mm.</i> | <i>gammas</i> |                 |                    |
| May 14                    | 75          | 34-35      | 10            | 25              |                    |
| 18                        |             |            | 10            |                 |                    |
| 22                        |             | 37-38      | 10            | 22              | 3 died             |
| 26                        |             |            | 10            |                 |                    |
| 30                        |             | 40-42      | 10            |                 |                    |
| June 2                    | 94          |            | 10            |                 |                    |
| (Metamorphosis occurring) |             |            |               |                 |                    |
| 9                         |             |            | 10            |                 |                    |
| 13                        |             |            | 10            |                 |                    |
| 17                        |             |            | 10            |                 |                    |
| 22                        |             |            | 10            |                 |                    |
| 26                        |             |            | 10            |                 |                    |
| 29                        |             |            | 10            |                 |                    |
| July 2                    | 124         | 45-48      | Total 120     | 12              | 10 cases preserved |
| 6                         |             |            | 10            |                 |                    |
| 9                         |             |            | 10            |                 |                    |
| 13                        | 135         | 45-50      | Total 150     | All preserved   |                    |

metamorphosis. Growth occurred slowly but uniformly as evidenced by the fact that all members of the group came to metamorphosis within a span of a few days. The essential data are given in table 1.

#### CONTROLS AND CONDITIONS WITH RESPECT TO SEX DIFFERENTIATION AT THE BEGINNING OF THE EXPERIMENT

Two types of controls were carried throughout the experiment, one of which received equivalent injections of the solvent alone, while the other group were uninjected. In addition,



controls were examined histologically prior to the experiment in order to determine how far the process of differentiation had advanced, and the sex ratio prevailing in the early stages of differentiation. Nine control specimens preserved some time before the start, at a length of 27 mm., proved on histological examination to be undergoing early differentiation. There were 3 ♀, 4 ♂ and 2 not clearly differentiated. The latter probably would have developed as males. Seven other specimens preserved just at the time the experiment began, were all rather well differentiated: 4 ♀, 3 ♂. Under the conditions stated, therefore, this stock showed a normal sex ratio (7 ♀ : 7 ♂), with differentiation occurring somewhat later than usual because of the slower rate of development in general.

#### RESULTS OF THE ADMINISTRATION OF ESTRONE

##### *1. Gross examination*

At autopsy there were no external evidences of hormone action. The cloacae appeared identical with those of control specimens, as was to be expected. Experimental animals were distinguishable from uninjected controls only by a slight translucency of the body wall, due presumably to a clearing action of the oil on the living tissues, a condition present also in controls injected with oil alone. On opening the body cavity a small residue of oil could usually be expressed.

After dissection and removal of the gastro-intestinal tract the parts of the urino-genital system are clearly displayed. The kidneys then appear as elongate band-like structures on either side of the midline, traversing the posterior two-thirds of the body cavity. Along the lateral border of each kidney lies a slender but dense white cord containing the gonoducts. Posteriorly these cords terminate in the cloacal wall; anteriorly they pass from the ends of the kidneys onward to the limits of the body cavity. In the posterior half of the body a cord consists only of the mesonephric or wolffian duct and its investment of connective tissue, covered by the reflected peritoneum. It is less prominent in this region because of

close proximity to the kidney. The müllerian ducts are incomplete and not sufficiently developed to be grossly separable from the mesonephric ducts. They exist only in the anterior half of the body cavity. These conditions as they pertain to control specimens are illustrated in figure 1. The ratio in nine controls was again normal: 5 ♀, 4 ♂.

The gonads are attached to the ventro-mesial surfaces of the kidneys near their anterior ends. Ordinarily in animals after metamorphosis ovary and testis are sharply differentiated and identification may be made at a glance. Ovaries are comparatively large, irregularly lobate organs beginning to be folded or convoluted along their free borders. The surface appears coarsely granular due to innumerable rounded elevations produced by the growing follicles (fig. 1, right). Testes are small and fusiform, with a relatively smooth surface, typically stippled with a fine pigment which is hardly visible without considerable magnification (fig. 1, left). This is the appearance of typical controls at metamorphosis or in the early post-metamorphic period.

The gonads of the experimental group notably lacked the sharp differentiation of normal gonads. Intergrading sizes were present and a majority undoubtedly resembled ovaries. Since no important differences developed between the 50-day and the 60-day groups, all will be included in one description. Grossly three types could be tentatively recognized. Gonads of the first type were unmistakable ovaries, quite indistinguishable from the normal. Others, while retaining the character of ovaries, gradually diminished in size, to intergrade closely with smaller sizes, difficult to classify certainly with the unaided eye. Under the binocular dissecting microscope most of the smaller gonads also resembled rudimentary ovaries at an earlier phase of differentiation, and would ordinarily be classed as such. Gonads of this type finally merged with still smaller forms of which no more than three could be called testis-like in character. The twenty-two experimental subjects receiving estrone, on the basis of gross examination, were therefore classifiable as follows: twelve were sufficiently characteristic and of a size as to be indistinguishable from female

controls (these may all be regarded as genetic females and the ovaries in fig. 1 faithfully represent their appearance); seven were ovarian in character, and while definitely underdeveloped approached by degrees the proportions of normal ovaries, which they also resembled in form. Three ovaries of this type are shown in figure 2. Only three were testis-like in character, two of which are shown in figure 3 and, in view of the intergrading forms described, required histological study before final identification. Clearly there is here a large preponderance of gonads of definitely ovarian character. Since, as we have seen, controls examined histologically before the experiment showed a normal sex ratio, and controls of the 60-day group at the termination of the experiment were again typical as to sex ratio and type of differentiation, the appearances described were considered significant.

## *2. Results of histological study. The gonads*

Histological study of complete serial sections confirmed in every respect the classification based on gross examination, and again members of the 50- and 60-day groups showed such slight differences as to warrant including both in one description. Of the first group of twelve, indistinguishable in no way from ovaries of the control group, several were sectioned and found to be typical in every respect. The section of a normal ovary in figure 4 is representative. These may be regarded as the expected contingent of genetic females and apparently are not affected by a homotypic hormone. It is evident that the hormone has not been able to accelerate female development beyond the normal rate.

Seven cases comprised an intermediate group, typically or largely ovarian in external form, but markedly undeveloped as compared with normal ovaries. As a group they intergraded in size with testis-like gonads. All were studied histologically. Three proved to be small ovaries but with evidences of testicular origin (fig. 5). The cortices were generally rudimentary and limited to the distal aspect of the ovarian sac.

Numerous free germ cells lying in the sacs also indicate their probable derivation from testes by reversal. Four cases, however, although the ovarian phase is distinctly in the ascendancy, show prominent stages of transformation from testis to ovary, displayed locally at many points throughout the gonad (figs. 6, 7 and 8). The cortex does not completely invest the ovarian sac but is displaced distally, having the position of the cortical rudiment of the testis. The sac is small, thick-walled and otherwise atypical and numerous gonidia of medullary origin occur free within the cavity. Medullary masses at the hilus are common (figs. 6 and 7). There can be little doubt of the impending transformation of these intersexual cases into small ovaries identical with those of the preceding group. It is therefore reasonable to classify all the members of this morphologically intermediate group as ovaries retaining in varying degree the histological evidences of testicular origin.

The last group is composed of three cases already mentioned (fig. 3), in which the size and external form of the gonads were so testis-like as to raise little question of their identity. These gonads are indeed testes, relatively immature in their differentiation but with signs of early reversal apparent locally in every gonad (figs. 9 and 10). Indications of reversal consist chiefly in modifications of the rete cords and their relation to the germinal elements of the gonad. In normal testis development the rete cords proliferate centrally as branching strands which rapidly envelope the more proximal germ cells, thus giving rise to a compact proximo-central mass, the medulla, in which gonidia and rete elements are rather uniformly mingled (compare Burns, '35, figs. 2 and 3). Peripherally, a few gonidia usually remain as a fairly distinct zone or layer immediately adjacent to the peritoneal covering of the gonad. This zone is the cortical component, visibly differentiated to a degree in almost every testis, but transient in normal development. In reversal, the rete cord of a testis has its differentiation first checked and then modified so that it assumes the form of an expanding vesicle—the ovarian sac.

Thus many or most of the indifferent gonidia may be excluded from the rete apparatus at an early stage, remaining between the ovarian sac and the peritoneal coat where they become organized as cortex. Gonidia already appropriated by the rete cord during the testicular phase of its development, are now found lying loose within the ovarian sac, frequently in very large numbers (figs. 5 to 8). Such abandoned gonidia ultimately degenerate leaving a considerable cellular detritus, often observable for some time (Burns, '35). The three modified testes of this group show locally the early changes in the rete apparatus preparatory to assuming an ovarian

TABLE 2

|                         |                               | SEX RATIO |      | UNDIFFERENTIATED |
|-------------------------|-------------------------------|-----------|------|------------------|
|                         |                               | 7 ♀       | 7 ♂  | 2                |
| Beginning of experiment | Controls                      |           |      |                  |
|                         | Experimentals (assumed ratio) | 12 ♀      | 10 ♂ |                  |
| End of experiment       | Experimentals                 | 19 ♀      | 3 ♂  |                  |
|                         | Controls                      | 5 ♀       | 4 ♂  |                  |

form. The cellular constituents of the rete are aggregated in the central region of the gonad, where the arrangement frequently portends an ovarian sac, and in many cases lumen formation is actually beginning (for comparable stages of transforming testes in parabiotic pairs, compare plates 1 and 2, Burns, '35).

The histological identification of sex in the twenty-two members of the experimental group therefore establishes a secondary ratio of 19 ♀, seven of which are clearly of testicular origin, and 3 ♂, as opposed to the normal 1:1 ratio among controls determined both at the beginning and the end of the experiment. The results may be presented as in table 2.



### 3. *The gonoducts*

*a. Normal conditions as determined in controls.* As stated earlier, the gonoducts on either side are closely invested in a slender cord of an opaque white color, lying along the lateral border of the kidney. From the anterior ends of the kidneys the two cords pass forward to the limits of the body cavity. In this part of their course they lie closely parallel, separated only by the width of the vertebral column, which space is occupied by the aorta and the posterior cardinal veins (fig. 1). In the stages of development dealt with here, the ducts of control specimens are simple and unconvoluted; the cord is therefore almost perfectly straight.

The ducts are not equally developed at this time, however. While the mesonephric ducts (wolffian ducts) extend the full length of the body cavity, terminating at the cloaca, the oviducts (müllerian ducts) are as yet quite rudimentary, usually extending no further than the middle of the coelom. This can be ascertained only in sections. There is much individual variation in the length of these ducts, but no clear sexual dimorphism. Ten control specimens equally divided as to sex showed the developing oviducts ending at various levels from a point just anterior to the mesonephros to the posterior pole of the gonad. Furthermore, variation may be almost as great between the two sides of one individual. There is also variability in diameter of the rudimentary ducts and in development of the lumen, which may be fairly well formed locally while imperfect or absent at other nearby levels. The appearance of the lumen apparently does not uniformly follow an antero-posterior sequence. Figure 11 is an average cross section through the cords containing the gonoducts in a control at the end of the experiment—that is, shortly after metamorphosis. The level is a point halfway between the anterior ends of the kidneys and the anterior limit of the body cavity. The oviduct (müllerian duct) lies distally or laterally in the cords, the wolffian duct proximally. The former as a developing structure is now the larger of the two; the latter is retrogressing. The oviduct at the right

in figure 11 shows a well-defined lumen and a dense wall; the other is less developed and has no lumen.

The wolffian ducts have their origin, of course, as pronephric (segmental) ducts and as such were functional tubes extending the length of the body cavity, prior to and during the development of mesonephric units. At the stages under consideration they serve as mesonephric ducts. Anterior to the kidney, however, they still extend forward to the original pronephric level at the anterior limits of the body cavity, but only as slender epithelial cords of a vestigial character. Sections are required to positively establish their presence and in normal development this part of the duct soon disappears altogether. Of the vestigial wolffian ducts only that at the left is clearly visible in figure 11. It still retains a small lumen not usually persistent in these controls.

*b. Experimental modifications.* In all but a few experimental animals there is macroscopically a distinct thickening of the longitudinal cord containing the gonoducts, more readily observed because of the whiteness of the cord (fig. 2). Histologically, the effect is striking and is found to be due to a response of the oviducal component of the cord. This rudimentary duct not only extends farther posteriorly on the average than in controls, but is markedly greater in diameter. The latter is evidenced by a considerable increase in the size of the epithelial tube and lumen, and by a heavier connective tissue investment in which tissue of mucoid type is commonly found. These features are shown in figure 12. This effect occurs equally in experimental subjects of either sex.

The greater extent of the oviducts posteriorly in experimental animals is well shown in figures 4 to 10. Figure 4 is from a control specimen at the middle of the gonad and figure 5, although actually from an exceptional injected larva, shows a condition of the oviduct at the same level, not appreciably greater than control development (anteriorly these ducts were heavier than controls, however). Therefore, at this level they may be permitted to represent the control condition.

Figures 6 to 10, inclusive, show the usual development of the oviducts at gonad levels in experimental animals, each representing a different subject. Thus is demonstrated a capacity of the rudimentary oviduct, irrespective of sex, to respond to estrone to a marked degree; although in comparing this response with the events of normal development the fact that a relatively large dose was used is important.<sup>3</sup>

#### REMARKS

An extended discussion of these results will be deferred for the present in view of the fact that other experiments of the same type, including the administration of crystalline male hormones, are being prepared for publication. It may be pointed out now, however, that the demonstrated effects of estrone in larval *Amblystoma* are quite in keeping with the work on the developing chick and the young alligator already referred to, and with the results on sex reversal in *Amblystoma* obtained by means of the parabiosis technique and the grafting of embryonic and larval gonads. The mechanism of action, as reflected in the histological picture in transforming gonads, appears to be the same. The stimulatory effect on the oviduct is demonstrated much earlier than in other work on *Amblystoma*, but this is probably attributable to a higher concentration of the hormone than obtains under the more natural conditions of hormone production following grafting or parabiosis. Clearly the results argue for a close similarity if not identity of the sex differentiating substances in mammals, sauropsids and amphibians.

As in the chick, the action of the hormone is specific, the development of female characters being decidedly favored. The manner in which these effects are produced—that is, the mode of action of the hormone—is of interest. With reference to histological transformation in the gonad, the question cannot be answered with finality, but from the close resemblances

<sup>3</sup> The findings in the experimental animals of this group are confirmed so far as the changes in the gonads are concerned by a parallel experiment carried out on larvae of *A. tigrinum* by Messrs. Stanley Leavey and Richard J. Ackart, in this laboratory. A study of the ducts has not yet been made in this material, however.

shown, it appears that the hormone acts in each instance on the rete apparatus, directing its development toward the female type. This in effect inhibits medullary development, and so indirectly favors the differentiation of cortex. This is the manner of action generally accepted for amphibian sex reversal.

It is noteworthy that while differentiating testes have their development reversed by the action of estrone, there is no evidence that the ovaries of genetic females are in any way accelerated in their development. This may be merely because cortical development in genetic females is already proceeding at capacity; however, it is possible to regard it as evidence that the hormone does not act directly on the cortex itself. If cortical development is a consequence of differentiation of the rete apparatus as an ovarian sac, in the ovary of genetic females this condition already obtains and the possibilities are fully realized. The case is different for the rudimentary male cortex held in restraint by medullary development. If we conceive that the hormone suppresses medullary development through its action on the rete apparatus, the response of the cortex might then be in proportion to this suppression, but in theory could never surpass the limits represented by normal female development. This is an attractive view compatible with the evidence, but cannot be regarded as fully proven.

The action on the oviduct, on the other hand, is clearer. The hormone acts positively and directly, since the growth of this structure appears unrelated to the state of the gonad and may considerably exceed the best normal development. This growth is as great in larvae possessing extremely rudimentary ovaries of testicular origin, or even slightly modified testes, as in genetic females with normally developed ovaries. Moreover, there is marked precocious development of the oviducts in experimental females whose ovaries show no evidences of stimulation. Finally the entire period covered by the experiment is well within that phase of normal development in which the gonoducts undergo self differentiation independently of the gonads.



In concluding we may point out that in this experiment there is no indication of the 'paradoxical effect' reported by Padoa in tadpoles, in which follicular hormone actually induced the differentiation of males only, a phenomenon not yet explained.

#### SUMMARY AND CONCLUSIONS

1. Repeated injections of small doses of estrone into larval *Amblystoma punctatum* beginning shortly after the onset of sex differentiation in the gonads, causes transformation of the testes of genetic males into ovaries. The process of reversal takes the same histological course as has been described following parabiosis and grafting of the embryonic gonad. Comparatively few testes retain more than traces of their original character. The development of ovaries, however, is not appreciably accelerated.

Controls studied histologically both at the beginning of the experiment and again at its conclusion, showed the normal 1:1 ratio of the sexes, with no unusual manifestation of larval bisexuality.

2. The developing oviducts of normal larvae during the period covered by the experiment are both incomplete and rudimentary in their differentiation. There is as yet no sexual dimorphism. In this state of immaturity oviducts are nevertheless capable of a precocious response to estrone in the dosage given. The response occurs equally in both sexes and involves an increase in both length and diameter. It is independent of conditions in the gonads and may therefore be attributed to a direct action of the hormone.

3. No effect on the wolffian ducts was observed and it is concluded that these prospective male structures are indifferent to the presence of estrone. The same is true for the cloaca insofar as visible growth of the organ is concerned. The cloacal glands have not been fully examined histologically and an exception might perhaps be expected in the case of the spermathecae.



4. So far as this experiment goes estrone behaves in larval *Amblystoma* as in the developing chick, specifically promoting cortical growth in the gonad and accelerating development of the female ducts in larvae of both sexes, but has no demonstrable influence on male parts.

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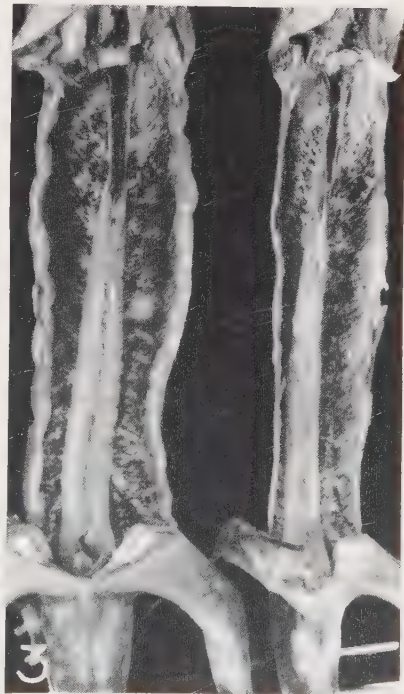
## PLATE 1

### EXPLANATION OF FIGURES

1 Control specimens at the end of the experiment. The testes of the two males (left) are slender fusiform bodies attached to the kidneys near the midline. The white cord-like structures lying along the lateral borders of the kidneys and extending forward to the limits of the body cavity, contain the gonoducts. The cloacae are sexually indifferent.  $\times 5$ .

2 Experimental subjects. Three members of the intermediate group, showing undeveloped or rudimentary ovaries derived from testes. In size they approach testes, but under higher magnification show many of the characteristics of ovaries. Note that the cords containing the gonoducts are definitely heavier in two of the three cases. The cloacae show no change in appearance.  $\times 5$ .

3 Two of the three cases possessing gonads of male type. Grossly these were classified as male, but histologically were becoming intersexual. The cloacae are not modified.  $\times 5$ .



## PLATE 2

### EXPLANATION OF FIGURES

Figures 4 to 12, inclusive, are photomicrographs.  $\times 80$ .

4 Section through the middle region of the ovaries of a female control. Large growing oocytes predominate, obliterating the ovarian cavity. At the lateral border of the mesonephros on the right is the wolffian duct. The müllerian duct is hardly visible along the lateral wall of the wolffian duct.

5 Experimental specimen of the intermediate group (compare fig. 2) showing rudimentary ovary derived from a testis. Note the gonias of medullary origin lying in the ovarian sacs. The müllerian duct (left side) is but slightly affected. For details, see text.

6 Rudimentary ovary of another member of the intermediate group, showing persistent medulla. The müllerian duct of this specimen is markedly developed.

7 Another ovary of the intermediate group with gonias in the ovarian sacs, and other evidences of testicular origin. Note the medullary rest in the hilar region of the ovary at the right, and the development of the müllerian ducts. For details, see text.

8 Gonads of a fourth member of the intermediate group, showing the same features.







### PLATE 3

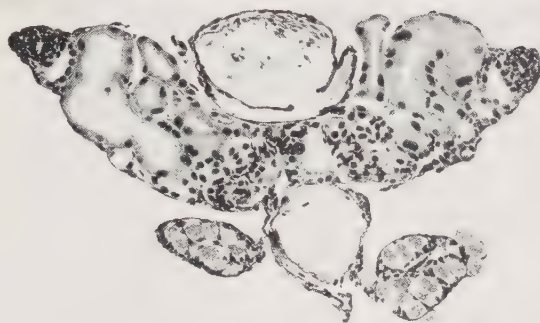
#### EXPLANATION OF FIGURES

9 and 10 Two of three members of the experimental group having gonads superficially of male type. The sections show incipient stages of transformation to ovaries with the appearance of central cavities. For details, see text. Note the development of the oviducts.

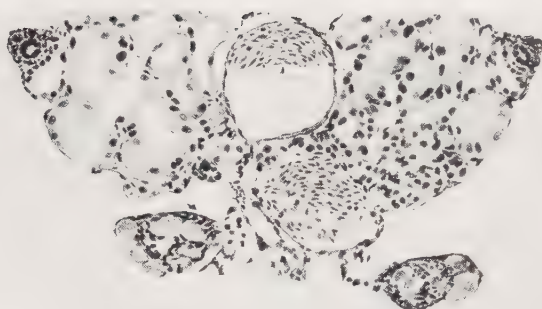
11 Section from a control across the cords containing the gonoducts, at a level anterior to the kidneys. Each cord contains the developing oviduct distally and the retrogressing wolffian duct (pronephric portion) proximally. The oviducts are rudimentary, the wolffian ducts reduced to slender epithelial strands.

12 Corresponding section from a typical experimental specimen, showing effect of hormone administration on the oviducts at this anterior level. For details, see text.

9



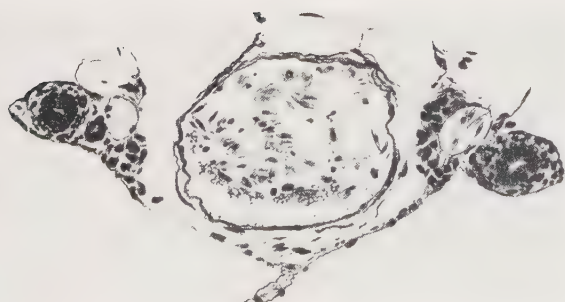
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## INCOMPLETE DEVELOPMENT OF THE DIAPHRAGM IN MICE CAUSING DEATH FROM ASPHYXIA

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### THREE FIGURES

Among the colony of mice kept in the department of anatomy of the Peiping Union Medical College, it was observed that occasionally newborn mice died soon after birth with greatly distended abdomens and lilac cyanosis of the skin. Altogether thirteen of these specimens were collected and preserved, and the cause of death investigated.

No literature on this abnormality could be found, but it became known that similar cases occur in other mouse colonies and that sometimes the cause of death is attributed to the presence of harelip. The mice, while sucking, are supposed to swallow large quantities of air which balloon the intestinal tract, possibly rupturing it, and the air causes pressure on the diaphragm and distension of the abdomen. Death would then take place from asphyxia.

Careful examination of our specimens under a dissecting microscope showed no evidence of harelip. Also, it seemed fairly definite under the microscope that no part of the alimentary tract was distended. Consequently, a scheme of investigation was drawn up to discover the cause of the distension.

Examination of cross sections showed that the whole of the abdominal part of the alimentary tract was collapsed, there was no evidence of distension of the tract or of rupture

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in any part of it. Apparently then, gas had collected in the abdominal cavity from some source other than the alimentary tract. Two experiments were now carried out as follows:

*Experiment 1.* A living normal newborn mouse, was taken and air slowly introduced into the peritoneal cavity by puncturing the left lower quadrant of the abdomen with a fine needle attached to a small hypodermic syringe. With a total of 1 cc. of air in the cavity, respiratory distress was marked and respiration ceased in approximately 10 seconds.

Autopsy of the mouse showed that death had taken place from asphyxia. The skin was a lilac color; the lungs were collapsed, the heart and the large veins were engorged with dark blood; the intestines were pressed together and the liver displaced upward.

*Experiment 2.* An artificial harelip was made in a normal newborn mouse and it was replaced with its mother. Distension of the abdomen did not follow, milk was seen in the stomach as a small white spot in the left hypochondrium, and development progressed normally.

Soon after these experiments, we were fortunate enough to obtain a litter of eleven full-term mice. Two were stillborn, eight were apparently normal, one showed some distension of the abdomen but was still alive. The abnormal mouse was carefully examined for the presence of harelip. This was not found. The mouse was then replaced with its mother and kept under regular observation. After about 1 hour, the abdomen was tightly distended, respiration was jerky and spasmodic, and the normal pink color of the skin was darkening; 0.5 cc. of gas was removed from the peritoneal cavity by means of a fine needle inserted into the left lower quadrant of the abdomen. The gas was kept for analysis. With the relief of the distension, respiration soon returned to normal and the color of the skin improved. Two hours later, distension was again marked, and on this occasion, milk in the stomach was plainly visible as a white spot in the left hypochondrium; 0.8 cc. of gas was removed from the peritoneal cavity and added to the previous specimen obtained. Three hours after



the second paracentesis, a further 0.8 cc. of gas was removed and stored. Three and one-half hours later, a distension was again very marked; the stomach was half full of milk; 1 cc. of gas was taken out but on this occasion, respiration did not improve, the lilac color of the skin persisted and the mouse died.

Autopsy showed that death had occurred from asphyxia, the lungs being collapsed, the heart and large veins engorged with dark blood; the stomach was small and it contained a little milk; the peritoneum appeared normal and there was no free fluid in the cavity; the intestines were compressed, no rupture could be found; finally, the liver was displaced upward under the hyochondria. Analysis of the gas collected, was very kindly carried out by Dr. Hsien Wu of the department of biochemistry. He stated that the sample contained a little more carbon dioxide and little less oxygen than ordinary air.

The results of the above experiment suggested that there was an abnormal one-way passage between the thoracic and abdominal cavities. We had subsequent opportunities to confirm this.

Another mother gave birth to a full-term mouse during the night. It was found dead in the morning, blue in color and with a greatly distended abdomen. Harelip or any other visible abnormality was not present. The gas was removed from the abdominal cavity, then a stream of air was gently forced into the oral cavity. Almost immediately, the abdomen became distended with gas, and the distension could not be reduced by pressure on the abdominal wall. The specimen was preserved and prepared for serial cross section.

A few weeks later, a similar specimen was obtained. In this experiment, the dead mouse was beheaded and a fine hollow glass tube inserted into the trachea. The abdominal cavity was then laid open, the gas escaped and it was seen that the stomach and intestines were compressed, the liver hidden away under the hyochondria. A gentle stream of air was now passed down the trachea. Almost immediately,

the liver moved down slightly and a bubble of air became visible in the right hypochondrium. In the left hypochondrium, the stomach remained collapsed, and no air bubble appeared. The next step was to introduce India ink into the trachea instead of air. The ink trickled through the lung tissue and eventually appeared in the peritoneal cavity in the same region where the air bubble had appeared, i.e., the right hypochondrium, in the region of the right crus of the diaphragm. This seemed to prove conclusively that the right pleura and the right half of the diaphragm were deficient, allowing air from the right lung to pass into the abdominal cavity.

In order to complete the investigation, serial cross sections of some of the abnormal mice were examined, with particular attention paid to the diaphragm. In each case, the findings were essentially the same, therefore only two specimens will be described and illustrated.

In the specimen illustrated by figure 1 the diaphragm shows a membranous portion which had ruptured (fig. 1 at x). A large amount of air had collected in the abdominal cavity, compressing the various organs. For the liver this is visible in figure 1.

In the other specimen (figs. 2 and 3) that portion of the diaphragm which corresponds with the embryonic left pleuro-peritoneal membrane has failed to develop its musculature completely. The dome of the diaphragm lies at a higher level than is normally the case. There is massive collapse of the lung tissue. The membranous part of the diaphragm and the pleura of the left lung are stripped up, allowing the lung tissue to be in direct contact with the liver. Consequently, air from the lung can pass directly into the peritoneal cavity through small ruptures in the lung tissue.

As regards the mechanism whereby the air is imprisoned in the cavity, this is a matter of conjecture. We suggest that the liver acts as a valve. The liver accurately fits the dome of the diaphragm. During inspiration, the diaphragm contracts and moves downward onto the liver and the deficiency in it

is occluded by the latter. During expiration, the diaphragm relaxes. Air in the lung can then pass through the deficiency and so gain the peritoneal cavity, where it remains as it cannot escape during inspiration. The volume of air increases with every expiratory movement and when this volume approaches 1 cc., the pressure it exerts on the liver and diaphragm greatly embarrasses inspiration with the result that the mouse rapidly dies from asphyxia. When it is recalled



Fig. 1 Part of a transverse section through a newborn mouse. The diaphragm, D, shows a muscular and a membranous part, the latter being broken at x. L, lung, Li, liver. The space below the liver was filled with air during life.

that analysis of the gas found in the abdomen showed that its composition was very similar to that of expired air, this theory seems to be the correct explanation.

The colony in which these abnormal mice were born was kept for research on problems of genetics. Dr. A. B. D. Fortuyn kindly supplied me with the following information from his records. The mice in the colony came from three independent sources and in only one of the three groups, albino *Mus musculus* imported from the U. S. A., abnormal mice of this type were found, approximately one in 2000

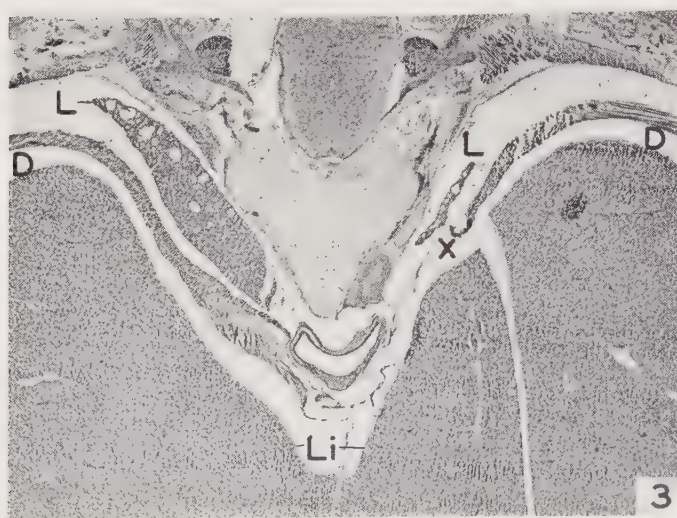
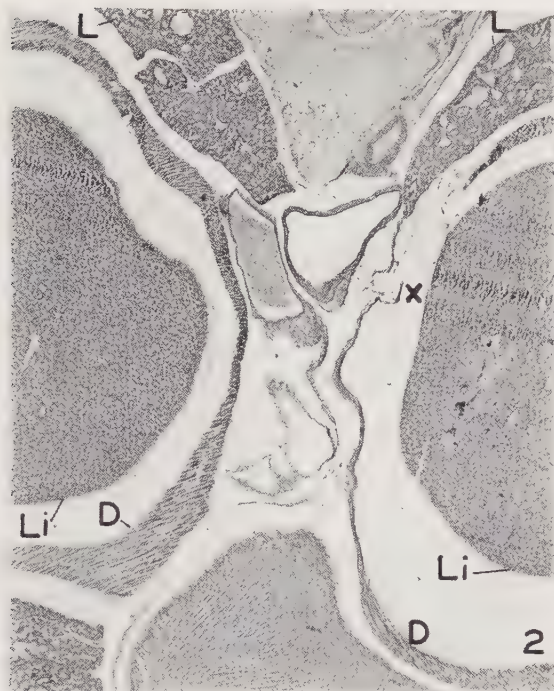
newborn mice. One case was observed in a strain where no special form of inbreeding was practiced, while the other twelve were in groups where sister-brother inbreeding had been going on for twelve to thirty-three generations. The mothers of all abnormal young were known to be very distantly related. The two nearest related mothers belonged to the thirty-second and thirty-third inbred generation and they had one and the same pair of ancestors in the twenty-seventh generation. Heredity, therefore, seems to play a minor role in the genesis of this abnormality.

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Fig. 2 Part of a transverse section through a newborn mouse. The diaphragm forms two domes, one in each half of the body and in each of them a liver lobe (Li) extends. The right half of the diaphragm is muscular, the left half has a membranous part which is broken at x. Here lung (L) and liver (Li) may come in contact.

Fig. 3 This is part of a transverse section through the same mouse as that of figure 2 at a lower level. A portion of each lung (L) is visible, the liver (Li) forms more or less one mass. The right half of the diaphragm (D) is muscular and very different from the left half which is in part membranous and broken at x.









# GROWTH OF FOLLICLES IN THE OVARIES OF THE BAT MYOTIS LUCIFUGUS LUCIFUGUS

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FIVE FIGURES

## INTRODUCTION

The changes which take place in the ovaries during the reproductive cycle have been studied extensively in mammals, such as the rat and mouse, which have relatively short oestrous cycles. Evans and Swezy ('31) have recorded that during each cycle a considerable group of follicles begins growth. Some of these complete their differentiation and rupture; the majority undergo retrogression. In the mouse it seems clear that this periodic initiation of activity in the germ cells involves the germinal epithelium (Allen, '23; Allen and Creadick, '37).

In the bats of the temperate zone there is only one reproductive cycle each year. The species *Myotis lucifugus lucifugus*, which is a cave resident in Missouri during the winter (Guthrie, '33 a), follows the general pattern for such mammals. Ovulation is spontaneous and occurs sometime in April, whenever the females leave the caves. A single tertiary, or vesicular, follicle containing a small antrum is present in October at the onset of the cave-dwelling period and grows but little, if at all, until the preovulation period in the spring. Primary follicles, consisting of a single layer of squamous cells, and both unilaminar and multilaminar secondary or growing follicles<sup>1</sup> are conspicuous throughout the so-called

<sup>1</sup>The multilaminar secondary follicles correspond to follicle types 2 to 5 described by Pincus and Enzmann ('37) for the rabbit. They do not recognize the unilaminar secondary follicle, in which the squamous cells of the primary follicle have become cuboidal, as a type.

hibernating months. Insemination occurs in the autumn, but females apparently again become receptive to males in periods of activity as the winter advances. Sperm are phagocytized in the uterus and eliminated through the vagina during the winter (Guthrie, '33 b). The presence of the vesicular follicle and the evidence of recurrent inseminations before its rupture made it seem likely that these animals were in continuous oestrus from October to April (compare Caffier, '34; Caffier and Kolbow, '34). Granting this possibility, what would be the effect upon the growth of secondary follicles? Would a knowledge of the total follicle population be of value in clarifying our understanding of the reproductive cycle?

In order to answer these questions a census of all normal growing follicles and follicles in early stages of retrogression was made in both ovaries of a series of females in which a cytological study of the ovaries had been made (Guthrie and Jeffers, '38 a). Comparable observations have been made on ovaries of *M. grisescens* collected at intervals throughout the year and will be presented elsewhere.

#### MATERIAL AND METHODS

Ovaries of forty-five females collected at intervals of about 2 weeks from the end of October until late April were used. The amount of wear on the teeth, which affords a basis for comparing the ages of individuals, was observed with a hand-lens. Twenty of the animals were young, with no discernible wear on their teeth; eleven had an appreciable amount of wear on the teeth; and in fourteen the teeth were conspicuously worn. *M. lucifugus lucifugus* becomes sexually mature during its first year of life. In forty-three animals, a normal vesicular follicle was present; in two bats, collected in April, young corpora lutea were found.

The bats were killed by decapitation within 24 hours after they were collected and the ovaries placed in fixing fluid as soon as possible. All the ovaries used in this study were fixed in Champy's fluid (Lee, '37, p. 35) and mordanted in a mixture of 1% chromic acid and pyroligneous acid (2:1) and in

a solution of 3% potassium bichromate for 1 day each. Dehydration and embedding in paraffin were carried out in the usual way, using xylol as a clearing agent, and serial sections were cut at 3 or 4  $\mu$ . Sections were stained by Kull's acid fuchsin-thionin-aurantia method (Lee, '37, p. 306).

The smallest follicles included in the counts consisted of a single layer of cuboidal cells around an oocyte, that is, they were unilaminar secondary or growing follicles. Primary follicles were not considered. Two diameters of each follicle were measured and the average taken as the diameter of the structure; the theca was not included. Measurements were made at a magnification of  $\times 600$  with an ocular micrometer, each unit of which was equal to 4  $\mu$  at this magnification.

#### OBSERVATIONS

##### *Normal follicles*

The number of normal growing follicles in both ovaries of this bat varies in different individuals from 53 to 305, exclusive of the single vesicular follicle (table 1). The average number of follicles is 157. In 22.2% of the bats studied, less than 100 follicles are present; in 46.7%, between 100 and 200 follicles occur; and in 31.1%, over 200 follicles are found. In only one individual (2.2%) are there more than 300 follicles.

To find the relationship between the age of bats and the number of follicles in their ovaries, the forty-five animals were divided into groups on the basis of the amount of wear on their teeth. In the group of the youngest bats, with no wear on the teeth (twenty individuals), the number of follicles varies from 53 to 305 (average 182.3); in bats showing some wear on the teeth (eleven individuals), the variation is from 87 to 284 (average 168.7); and in the fourteen bats in which the teeth are conspicuously worn, from 65 to 173 follicles occur (average 110.1).

In order to determine whether there is a preponderance of follicles in a single ovary of the bat, the percentages of the total normal follicles occurring in the right ovary and in the

TABLE 1  
Counts of follicles in individual bats

| SERIAL<br>NO. OF<br>BAT | DATE OF<br>COLLEC-<br>TION | NUMBER OF FOLLICLES |                 |         |                  |         |                  |        |         | DIAMETER<br>OF VESICULAR<br>FOLLICLE<br>(MICRONS) |
|-------------------------|----------------------------|---------------------|-----------------|---------|------------------|---------|------------------|--------|---------|---------------------------------------------------|
|                         |                            | Normal              | Normal          | Atretic | Normal           | Atretic | Atretic          | Normal | Atretic |                                                   |
|                         |                            | 24 to 63 $\mu$      | 64 to 103 $\mu$ |         | 104 to 163 $\mu$ |         | 164 to 183 $\mu$ | Total  |         |                                                   |
| 598                     | 10-19-31                   | 68                  | 31              |         | 9                | 2       |                  | 108    | 2       | 345                                               |
| 614                     | 10-28-31                   | 85                  | 57              | 4       | 2                | 26      |                  | 144    | 30      | 358                                               |
| 1128                    | 10-31-34                   | 80                  | 47              |         | 14               | 8       | 1                | 141    | 9       | 305                                               |
| 640                     | 11- 8-31                   | 28                  | 21              | 2       | 4                | 4       |                  | 53     | 6       | 354                                               |
| 1131                    | 11- 9-34                   | 103                 | 83              | 1       | 28               | 30      |                  | 214    | 31      | 418                                               |
| 639                     | 11- 8-31                   | 96                  | 61              |         | 16               | 16      |                  | 173    | 16      | 359                                               |
| 1129                    | 11- 9-34                   | 130                 | 95              | 2       | 17               | 33      |                  | 242    | 35      | 348                                               |
| 1085                    | 11-10-33                   | 178                 | 94              |         | 15               | 22      |                  | 287    | 22      | 371                                               |
| 668                     | 11-21-31                   | 31                  | 16              |         | 10               | 5       |                  | 57     | 5       | 376                                               |
| 672                     | 11-21-31                   | 54                  | 45              | 5       | 9                | 22      |                  | 108    | 27      | 280                                               |
| 687                     | 12- 3-31                   | 54                  | 19              |         | 11               | 9       |                  | 84     | 9       | 308                                               |
| 689                     | 12- 3-31                   | 43                  | 20              | 1       | 2                | 6       | 2                | 65     | 9       | 279                                               |
| 710                     | 12-12-31                   | 67                  | 50              |         | 11               | 20      |                  | 128    | 20      | 322                                               |
| 734                     | 12-26-31                   | 43                  | 29              |         | 10               | 5       |                  | 82     | 5       | 373                                               |
| 736                     | 12-26-31                   | 121                 | 54              |         | 28               | 11      | 2                | 203    | 13      | 388                                               |
| 1157                    | 12-27-34                   | 86                  | 41              |         | 7                | 7       |                  | 134    | 7       | 350                                               |
| 738                     | 12-26-31                   | 167                 | 49              |         | 16               | 12      | 1                | 232    | 13      | 368                                               |
| 1159                    | 12-27-34                   | 183                 | 45              | 1       | 10               | 8       |                  | 238    | 9       | 394                                               |
| 1165                    | 1-14-35                    | 116                 | 27              |         | 1                | 13      |                  | 144    | 13      | 273                                               |
| 1170                    | 1-14-35                    | 133                 | 58              |         | 16               | 6       |                  | 207    | 6       | 359                                               |
| 756                     | 1-16-32                    | 55                  | 28              |         | 8                | 14      | 2                | 92     | 16      | 318                                               |
| 1090                    | 1-22-34                    | 194                 | 84              |         | 27               | 14      |                  | 305    | 14      | 400                                               |
| 1089                    | 1-22-34                    | 86                  | 26              |         | 11               | 6       |                  | 123    | 6       | 382                                               |
| 1092                    | 1-22-34                    | 154                 | 54              | 1       | 14               | 12      | 2                | 222    | 15      | 374                                               |
| 776                     | 2- 6-32                    | 114                 | 66              | 1       | 8                | 5       |                  | 188    | 6       | 342                                               |
| 778                     | 2- 6-32                    | 82                  | 57              | 1       | 23               | 17      |                  | 162    | 18      | 382                                               |
| 1183                    | 2-21-35                    | 78                  | 44              | 5       | 4                | 7       |                  | 126    | 12      | 328                                               |
| 1176                    | 2-21-35                    | 88                  | 34              |         | 7                | 10      |                  | 129    | 10      | 422                                               |
| 1175                    | 2-21-35                    | 103                 | 53              |         | 14               | 8       |                  | 170    | 8       | 388                                               |
| 830                     | 2-20-32                    | 53                  | 18              | 16      | 1                | 15      |                  | 72     | 31      | 402                                               |
| 1095                    | 3-10-34                    | 169                 | 91              | 2       | 8                | 22      |                  | 268    | 24      | 384                                               |
| 1100                    | 3-10-34                    | 145                 | 57              | 8       | 8                | 21      |                  | 210    | 29      | 324                                               |
| 1192                    | 3-14-35                    | 97                  | 32              | 6       |                  | 6       |                  | 129    | 12      | 337                                               |
| 1199                    | 3-14-35                    | 114                 | 39              | 4       | 28               | 11      |                  | 181    | 15      | 417                                               |
| 1191                    | 3-14-35                    | 56                  | 21              | 1       | 3                | 14      | 1                | 81     | 16      | *                                                 |
| 1193                    | 3-14-35                    | 43                  | 16              | 5       | 9                | 9       |                  | 68     | 14      | 355                                               |
| 1209                    | 3-29-35                    | 76                  | 48              | 5       | 5                | 10      |                  | 129    | 15      | 345                                               |
| 1214                    | 3-29-35                    | 112                 | 47              | 2       | 2                | 17      |                  | 161    | 19      | 388                                               |
| 1210                    | 3-29-35                    | 71                  | 29              |         | 1                | 9       |                  | 101    | 9       | 318                                               |
| 956                     | 4-16-32                    | 113                 | 45              |         | 15               | 5       |                  | 173    | 5       | 484                                               |
| 954                     | 4-16-32                    | 157                 | 72              | 8       | 12               | 8       |                  | 241    | 16      | 326                                               |
| 1224                    | 4-22-35                    | 197                 | 83              | 8       | 4                | 56      |                  | 284    | 64      | 375                                               |
| 1225                    | 4-22-35                    | 74                  | 49              | 2       | 5                | 31      |                  | 128    | 33      | 361                                               |
| 947                     | 4- 2-32                    | 128                 | 61              |         | 15               | 10      |                  | 204    | 10      | c.l.                                              |
| 1024                    | 4- 6-33                    | 52                  | 21              | 1       | 14               | 3       |                  | 87     | 4       | c.l.                                              |

EXPLANATION: Animals are arranged chronologically in the order in which they are plotted in figure 3. A space separates the groups collected at comparable dates. c.l., corpus luteum; \*, vesicular follicle damaged.



ovary containing the vesicular follicle were calculated for each individual. In 49% of the bats the vesicular follicle is in the right ovary.<sup>2</sup> The data on the number of follicles in a single ovary have been plotted (fig. 1), and it is seen from the graph that the follicles are not constantly more numerous in one ovary than in the other. The curves show that in a single animal as little as 39.6% or as much as 60.4% of the total follicular population may be present in one ovary. However, if

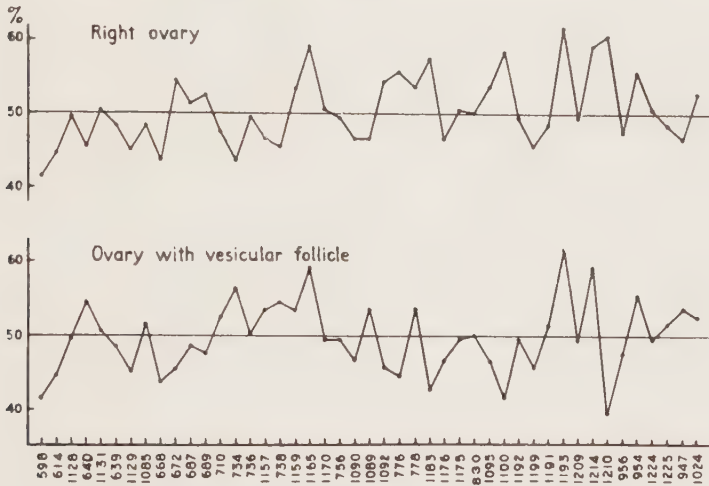


Fig. 1 Graph showing the percentage of normal growing follicles present in the right ovary and in the ovary containing the single vesicular follicle in individual bats.

the figures for the forty-five animals are combined, it is found that 49.5% of the total number of follicles in these animals is in the ovary with the vesicular follicle, while 50.8% is in the right ovary. In neither case is the deviation from a 1:1 ratio of statistical significance.

The bats were grouped together on the basis of the dates on which they were collected, and the average number of total

<sup>2</sup> While the vesicular follicle occurs as often in the left ovary as in the right, implantation in hundreds of *Myotis* observed is always in the right horn of the uterus. After the females reach sexual maturity the right horn is larger than the left throughout the year.

normal follicles calculated for each date. Figure 2 shows that the average number varies throughout the winter and spring months. The curve reaches peaks in early November, in late December and January, and again in late March and April. This curve is in many respects similar to that of the distribution of the smallest follicles measured (compare fig. 4).

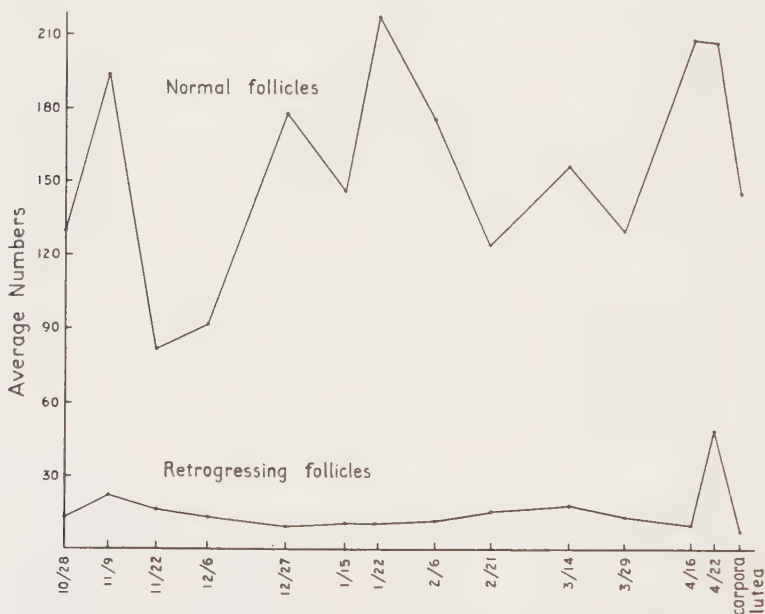


Fig. 2 Graph showing average numbers of all normal growing follicles and of follicles in early stages of retrogression in bats grouped in accordance with the dates of collection.

The normal secondary follicles in the ovaries of *M. lucifugus* range in size from 24 to 157  $\mu$  in diameter. These follicles were classified in four groups on the basis of size. The percentage of the total follicular population found in each group was calculated for each individual and the results plotted (fig. 3). The marked individual variation in the distribution of follicles of different sizes is shown by the graph.

The combined data for individuals killed at comparable dates are shown graphically in figure 4. The curve for fol-

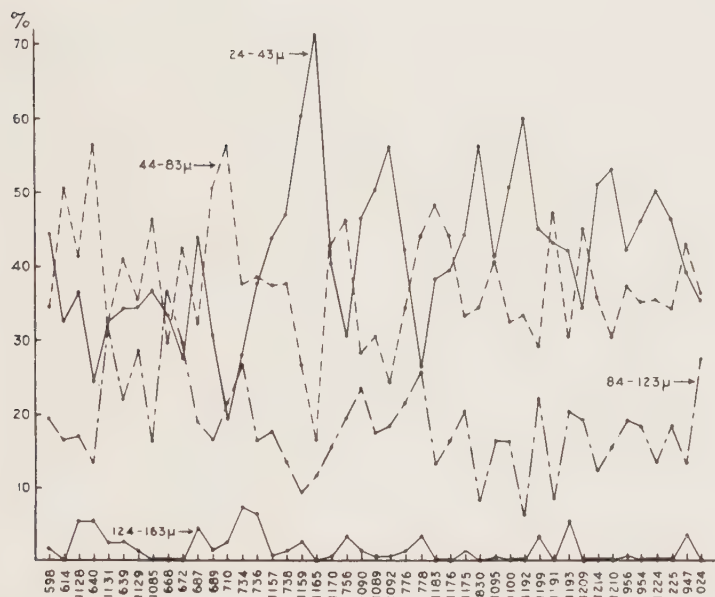


Fig. 3 Graph showing percentage of normal growing follicles in the different size groups in individual bats arranged in accordance with the dates of collection.

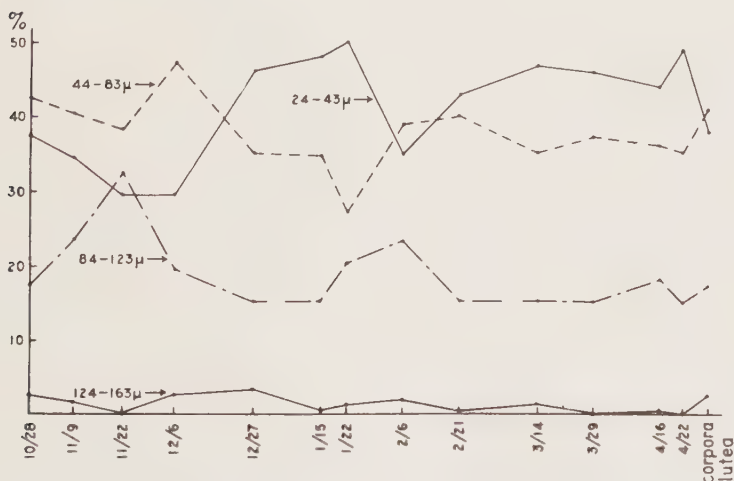


Fig. 4 Graph showing percentage of normal growing follicles in the different size ranges in bats grouped in accordance with the dates of collection.

licles 24 to 43  $\mu$  in diameter shows that there are periodic variations in the relative numbers of these follicles. They are very numerous toward the end of October, during late December and throughout January, and, again, during the last of February and most of March and April. In the two animals in which ovulation had occurred, there is a decrease in the number of these small follicles. The curve for follicles 44 to 83  $\mu$  in diameter also shows periodic variations. It has a conspicuous peak in early December, which we interpret as being conditioned by the peak in the curve of follicles 24 to 43  $\mu$  in diameter seen in late October. There is another rise in the curve in February, following the December and January increase in the smaller follicles. The remainder of the curve is without conspicuous variation. The curve for follicles 84 to 123  $\mu$  in diameter reveals two marked rises in the relative numbers of these follicles; the first occurs toward the end of November, the second is at its height early in February and follows the December peak in the curve for follicles 44 to 83  $\mu$  in diameter. The curve for the largest normal secondary follicles, 124 to 163  $\mu$  in diameter, indicates no marked variation in the relative numbers of these follicles, although the slight rise in December, following the November increase in follicles 84 to 124  $\mu$  in diameter may be significant.

The diameter of the vesicular follicle varies during the winter months from 273 to 422  $\mu$  (table 1). The largest follicle observed, from a bat killed April 16th (no. 956), had a diameter of 484  $\mu$  and contained an oocyte with a meiotic spindle. There is no indication that any marked growth of the tertiary follicle occurs until the period just before ovulation. The average diameter of seven vesicular follicles in November is 358  $\mu$  and of eight similar follicles in March is 358.5  $\mu$ .

### *Retrogressing follicles*

Follicles at all stages of degeneration occur in the ovaries of *M. lucifugus lucifugus* during the months when collections were made (see Guthrie and Jeffers, '38 a, for criteria of retrogression with stages and types). Only those in early

stages of retrogression of type I were measured and counted; their diameters have not yet decreased as a result of disintegration of the granulosa cells, and it is possible to determine how large they were when retrogression began. From 2 to 64 of these follicles are found in different individuals (table 1);

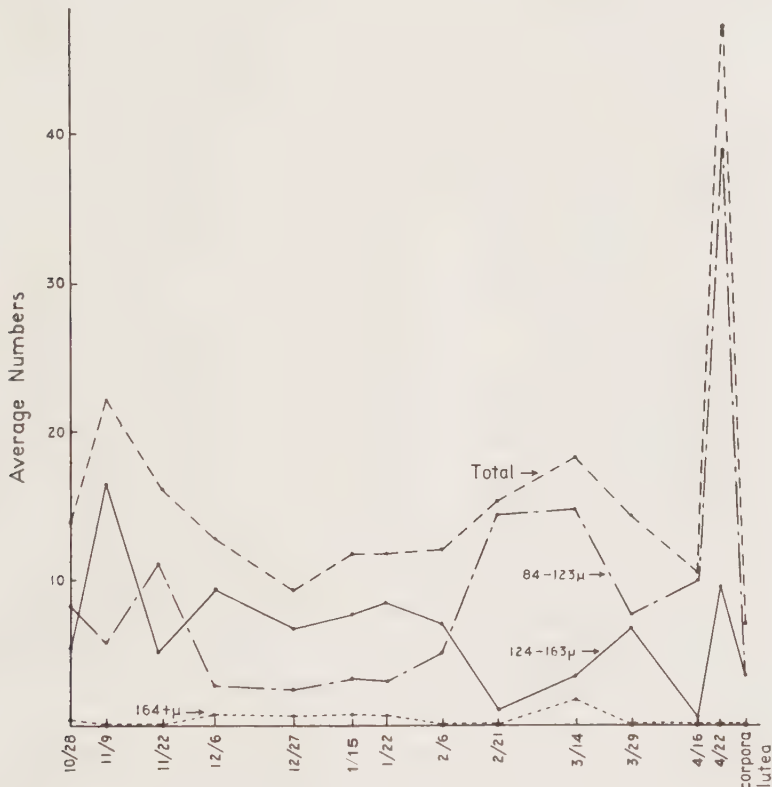


Fig. 5 Graph showing average numbers of all follicles in early stages of retrogression and of such follicles in different size ranges in bats grouped in accordance with the dates of collection.

the average number for the forty-five bats studied is 15.7. In 40% of the animals, 10 or less occur; in 37.8%, 11 to 20 are found; in 11.1%, there are 21 to 30; in 8.9%, 31 to 40 are present; and in only one bat (2.2%), do more than 40 such follicles occur.



The counts of follicles in early stages of retrogression for bats collected on comparable dates were combined, the average computed, and the data plotted (fig. 2). From the graph it is seen that, aside from the two individuals killed April 22nd in each of which atypical persistence of the vesicular follicle was noted, there is no pronounced variation in the number of degenerating follicles during the months in which collections could be made. However, slight increases in retrogression of follicles occur early in November and about the middle of March.

The size at which follicles begin to degenerate varies markedly. The smallest follicles in which degenerative changes are found in the granulosa are  $67\ \mu$  in diameter, the largest  $182\ \mu$ . Three-tenths per cent of the retrogressing follicles measured are  $64$  to  $83\ \mu$  in diameter;  $55.5\%$  are  $84$  to  $123\ \mu$ ;  $42.7\%$ ,  $124$  to  $163\ \mu$ ; and  $1.5\%$ ,  $164$  to  $183\ \mu$  in diameter.

If the average number of degenerating follicles of different sizes is calculated for bats collected at comparable dates (fig. 5) it becomes evident that from October until the middle of February approximately two-thirds of the follicles beginning to degenerate are  $124$  to  $163\ \mu$  in diameter; about one-third are  $84$  to  $123\ \mu$  in diameter. A few retrogressing follicles  $164$  to  $183\ \mu$  in diameter (a total of ten) are found during this period. After February 6th, there is a marked increase in the number of follicles  $84$  to  $123\ \mu$  in diameter that begin to degenerate and a decrease in the number of retrogressing follicles  $124$  to  $163\ \mu$  in diameter.

#### DISCUSSION

The number of normal follicles found in the ovaries of the bat is considerably smaller than that reported for the rat (Arai, '20; Swezy, '33 a), the mouse and ape (Blotvogel, '32), and the rabbit (Desaive, '35). However, for the analysis presented in this paper, only the secondary growing follicles were counted; the primary follicles and the tertiary follicle were not included. For this reason the counts given here are not comparable with those of most other investigators.

Arai ('20) reported that the ratio of the number of oocytes in the right ovary to that in the left was 1.03:1.00; for the entire series of rats, 50.6% of the oocytes occurred in the right ovary. For the forty-five *M. lucifugus lucifugus* studied, it was found that 50.8% of the normal growing follicles were in the right ovary.

In the rat, Arai ('20) determined by counting all the oocytes in both ovaries of thirty-nine individuals 1 to 947 days old that the number of oocytes decreased rapidly up to 20 days of age, after which the decrease was more gradual. The counts in *M. lucifugus lucifugus* suggest that a similar gradual decrease in the number of growing follicles occurs with age, but the number of individuals in each age group is small and the variation in the number of follicles is great.

Since only the growing follicles in the ovaries of the bat were counted, the data presented can throw light only on the growth of follicles, not on the seasonal variations in the proliferation of new oocytes, although differentiation of cells of the germinal epithelium is believed to occur throughout adult life (Guthrie and Jeffers, '38 a). Allen ('23) counted the mitoses in the cells of the germinal epithelium of the ovaries of mice at different stages of the oestrous cycle. A cyclic proliferation of the cells of the germinal epithelium, which had its peak at oestrus, was found. He regarded this as the first stage of ovogenesis. These results have been confirmed by Allen and Creadick ('37). Evans and Swezy ('31) reported that the proliferation of oocytes and the growth and degeneration of follicles were cyclic phenomena which corresponded in the rat, guinea pig and dog with the changes occurring during the oestrous cycle (compare Swezy, '33 b). While new sex cells were formed at all periods of the oestrous cycle, the proliferation of new oocytes was most marked during metoestrus, the number of growing follicles was at a maximum during anoestrus and at a minimum during oestrus. Myers, Young and Dempsey ('36) have reported that, in the guinea pig, follicles began to grow at all stages of the oestrous cycle. However, only those follicles beginning growth during a lim-

ited interval reached maturity and ruptured at the following oestrous period; all others degenerated. In contrast to these findings, the counts of normal growing follicles in the ovaries of *M. lucifugus lucifugus* indicate that, instead of a single wave of growth or a continuous initiation of growth in primary follicles, there are at least four peaks of follicular growth in this bat during the oestrous cycle. The curve for follicles 24 to 43  $\mu$  in diameter reveals three waves of growth in them during the 6 months studied (fig. 4). That these three are probably preceded by at least one other growth wave is suggested by the pronounced rise in the curve for follicles 84 to 123  $\mu$  in diameter late in November. The peaks of the three growth waves shown by the curve for follicles 24 to 43  $\mu$  in diameter occur at intervals of 60 to 75 days, and the peaks in the curves for follicles 44 to 83 and 84 to 123  $\mu$  in diameter appear at intervals of about 75 days.

It is possible, by means of the data plotted in figure 4, to estimate the rate at which follicles grow in *M. lucifugus lucifugus* during the period of so-called hibernation. The peak in the curve of follicles 24 to 43  $\mu$  in diameter for October 28th is followed in 45 days by a peak in the curve for follicles 44 to 83  $\mu$  in diameter. Similarly, about 45 days after the December and January increase in the number of follicles 24 to 43  $\mu$  in diameter there is an increase in the number of follicles 44 to 83  $\mu$  in diameter. A comparison of the curve for follicles 44 to 83  $\mu$  in diameter with that for follicles 84 to 123  $\mu$  in diameter shows that there is an interval of a little over 60 days between the corresponding peaks in the two curves. It requires, therefore, approximately 105 days for follicles 24 to 43  $\mu$  in diameter to become 84 to 123  $\mu$  in diameter, which is near the limit of size attained by follicles before they begin to degenerate.

No conspicuous wave-like variations in the numbers of degenerating follicles have been found to occur in the bat between October and the middle of April. However, it is interesting to note that there is a marked change beginning in February in the size attained by the secondary follicles before

they begin to degenerate. During the months from October to the middle of February, follicles may become relatively quite large before they degenerate; two-thirds of the retrogressing follicles are over  $124\ \mu$  in diameter. After the middle of February, the majority of the degenerating follicles are smaller than  $124\ \mu$  in diameter. It appears that something is limiting the growth of follicles beyond a certain size but not increasing the number of retrogressing follicles.

The reproductive cycle of the bat is known to have many peculiarities. First to be recognized were the facts of autumnal insemination and vernal ovulation. It has generally been held that the eggs are fertilized by sperm retained in the tract throughout hibernation (compare Hartman, '33). Observations on the tracts of many recently killed females (Guthrie, '33 b), the behavior of artificially ovulated eggs (Guthrie and Jeffers, '38 b), and the presence of abundant sperm in males of the species throughout the winter (Miller, '36, '37) have convinced us that, under the climatic conditions of central Missouri, spring copulations are the rule in these bats. Where the winters are more severe and temperatures more constant it seems possible that different reactions may prevail in the bats.

With the information accumulated over a period of years we may now formulate an explanation of the correlation of certain activities related to reproduction in the female bat. Since the cells of the pars distalis of the hypophysis of this bat have been shown to be understandable as stages in the differentiation of a single type of secretory cell (Guthrie, Jeffers and Sawyer, '36; Sawyer, '36) we shall assume that one secretion is produced by these cells. It is obvious that the preparation of however many hypophyseal extracts producing varied responses in several regions of the body does not establish that the active principles of such extracts exist as such in the intact gland (compare van Dyke, '36, pp. 174-175). Nor does the observation that a particular region, which is undergoing differentiation, seems to respond at one time to one fraction and at another time to a different fraction necessarily



suggest more than that cells are capable of different reactions and have different thresholds of response when in successive stages of differentiation. The effector cells are not static; the reaction is conditioned not only by the stimulus but by the effector (compare Maxwell, '34; Engle, '32, pp. 793-796). Lillie and his associates have made use of the concept of growth rate and hormone threshold in their brilliant analysis of feather patterns (compare Domm, Gustavson and Juhn, '32, pp. 636-637). Zuckerman ('37) has suggested that a variation in threshold to oestrogenic hormone is exhibited by the uterine endometrium of the monkey.

When females of *M. lucifugus lucifugus* enter the caves in the autumn there is a single follicle with a small antrum in the ovaries of each individual, the vaginal epithelium is cornified,<sup>3</sup> and the uterus contains sperm. These individuals are in a state of oestrus as judged by the criteria of vaginal reaction and receptivity to males. However, the uterus is not fully differentiated nor does ovulation immediately ensue. Let us assume that this state has been conditioned in the following way. During the summer, when these bats are active and feeding each night, follicles grow under the influence of the gonadotropic hormone of the hypophysis. At least one of these follicles<sup>4</sup> reaches the stage of differentiation at which an oestrogen is produced in a quantity sufficient to condition vaginal cornification and the response to males, but not to initiate differentiation in the uterus or tubes. These animals appear to correspond to Pfeiffer's ('35) 'single constant oestrous' rats, except that the mating reaction has been induced. There is nothing to suggest that progesterin has been responsible for the onset of receptivity, as Young ('37) has postulated for the guinea pig, since no luteinization of follicles is observed. We shall use the phrase 'submaximal oestrus'

<sup>3</sup> Statements concerning the condition of the reproductive tract are based on unpublished observations by Elizabeth M. Reeder, Ph.D. thesis, Univ. of Mo., 1938.

<sup>4</sup> We have no evidence that more than one tertiary follicle is produced in *M. lucifugus lucifugus* since we have no specimens taken at the season when several follicles with antra are found in the ovaries of *M. grisescens* (compare Guthrie and Jeffers, '38 a).



(Witschi and Pfeiffer, '35) to designate the condition of female bats which have entered the caves.

At the lower metabolic level of the early hibernating period (November) the gonadotropic hormonal output of the hypophysis is reduced and the average number of growing follicles is lowered while retrogression is conspicuous in the larger secondary follicles. The single tertiary follicle undergoes no detectable change, and retrogression of the secondary follicles is not accompanied by luteinization of the granulosa. It is interesting to note that Foster, Foster and Hisaw ('37) report a comparable manner of retrogression of follicles in hypophysectomized adult rabbits and find that small follicles with antra may persist and be capable of stimulation to grow for about 30 days after the removal of the gland.

By December the average number of growing follicles in these bats is greatly reduced, and the wave of retrogression is subsiding. One may assume that follicles growing at a certain rate require and take available hormone in a certain quantity. When the hormone has fallen below that level and many follicles have undergone retrogression the net result is a relative increase in the hormone available. The response to this is a gradual increase in growing follicles of the smallest size range. Such follicles have been stated by a number of workers to be independent of any hypophyseal influence as a result of studies on immature, growing animals or hypophysectomized adults. This matter is discussed in another place where the results of injection of hypophyseal extract upon these smallest growing follicles are presented (Guthrie and Jeffers, '38 b). Foster, Foster and Hisaw ('37) reported that a few primordial follicles persisted in the rabbit ovary for as long as 90 days after hypophysectomy. This observation, together with the long survival of follicles with small antra under the same circumstances, suggests that the total secretion of the hypophysis may not act directly on the gonad but through an intermediary. After hypophysectomy a low level of gonad stimulating substance from such a source maintains a limited number of follicles at certain stages of differentia-

tion for a considerable time. The smallest growing follicles are capable of response to very small quantities of this substance.

The number of smallest growing follicles in the bat undergoes a relative increase until about the middle of January when there is a rise in the percentage of larger secondary follicles which is conspicuous early in February. This is believed to be induced by an increase in the amount of gonadotropic hormone available, which is correlated with changing temperature conditions and an elevation of metabolic level in the bats as indicated by evidence of shifting populations at this time (compare Guthrie, '33 a). Along with this increase in number of larger secondary follicles the output of the oestrogenic substance increases and the females are again receptive to males in periods of activity. It is during this time that spontaneous ovulation under laboratory conditions begins (Guthrie and Jeffers, '38 b). By the end of February, however, the number of growing follicles has become too large for the amount of gonadotropic hormone available and retrogression is again on the increase, this time among smaller follicles. With the release of this drain on the supply of gonadotropic hormone the smallest follicles show a relative increase.

Toward the end of March, as the time of emergence from the caves nears, there is an increase in the average number of growing follicles again, but the tertiary follicle shows no definite trend toward increase in size so long as the females remain in the caves. We have by chance obtained one individual (no. 956) in which preovulatory growth has occurred. We believe that with an increase in metabolic activity the amount of gonadotropic hormone finally rises until the threshold of response of the tertiary follicle is reached. When this happens the tertiary follicle undergoes very rapid growth, and ovulation and swelling of uterus and tubes occur within 24 hours in many individuals removed from the caves at this time of year. This hypothesis that preovulatory growth and ovulation require a very high level of available gonadotropic hormone is consistent with the observation that the large

graafian follicles are the first to respond to diminution of the hormone in the hypophysectomized rabbit (Foster, Foster and Hisaw, '37), and that a large final intravenous injection of the follicle stimulating or of the luteinizing fraction of hypophyseal extract appears necessary in order to induce ovulation in the anoestrous cat (Foster and Hisaw, '35). Witschi and Pfeiffer ('35) used a large dose of the luteinizing fraction of the gonadotropic hormone in order to produce ovulation in their rats which were in submaximal oestrus.

In the bat there seems no reason to postulate the presence of more than one gonadotropic hormone if the possibility of differing thresholds of response in follicles at the several stages of differentiation be granted. There is no indication of any luteinization except in the granulosa of the follicle of ovulation. There is nothing which suggests to us that a swing from F.S.H. secretion to L.H. secretion occurs under the influence of an oestrogenic substance (compare Witschi and Pfeiffer, '35; Fevold, Hisaw and Greep, '36). A single gonadotropic hormone which is produced or becomes available in a limited quantity and for which follicles compete at all stages of growth but at different thresholds is adequate to explain the peculiarities and duration of the oestrous period in the bat. Evans and Swezy ('31) found for several mammals that the period when most follicles underwent retrogression was when a few completed the preovulatory growth. They found, also, that gestation did not inhibit the cyclic growth and degeneration of follicles, even though ovulation did not occur. Follicles growing during gestation sometimes luteinized in the rat but not in the guinea pig. Dempsey ('37) could find no effect of oestrogenic substance (progynon B, Schering) on rhythmicity of ovarian response in the guinea pig. The hibernating female bat is in submaximal oestrus as a consequence of an inadequate quantity of available gonadotropic hormone.

## SUMMARY

1. The number of normal growing follicles in the ovaries of *M. lucifugus lucifugus* ranges from 53 to 305; the average is 157 follicles, which are equally distributed in the two ovaries. The average number of normal growing follicles decreases somewhat with age.

2. The periodic variations in the relative numbers of follicles of different sizes which occur in the ovary during the months from October through April are interpreted as indicating follicular growth waves. Three peaks of growth are evident in follicles 24 to 43  $\mu$  in diameter, and these are followed by peaks of growth in the larger follicles. A fourth growth wave just prior to hibernation is suggested by the data.

3. Follicles in which retrogression is beginning vary in number from 2 to 64 in different individuals; the average is 15.7. From October until the middle of February, approximately two-thirds of the degenerating follicles are 124 to 183  $\mu$  in diameter; from February 21st on, the majority of the retrogressing follicles are 64 to 123  $\mu$  in diameter.

4. The four growth waves of the secondary follicles occur during a single oestrous cycle which terminates at the time of rupture of the one vesicular follicle present throughout the so-called hibernating season.

5. The hibernating female bat is in a state of submaximal oestrus conditioned by an inadequate quantity of a single gonadotropic substance.

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## THE EXTRUSIVE GROWTH AND ATTRITION OF THE INCISOR TEETH OF CAVIA COBAYA

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The continuous growth and the rapid attrition of the incisors of certain rodents were demonstrated by Fougereux (1770) and Oudet (1823). Subsequently, occasional observations were made on the weekly amount of growth and attrition. Addison and Appleton ('15) made some measurements of the weekly attrition of the white rat, and Dalldorf and Zall ('30), in the course of experiments on vitamin deficiencies, made some measurements on the guinea pig.

In 1929 the senior author began the study of the attrition of the incisors of rodents. The results of the investigation on domestic rabbits (Shadle, '36), and upon rats (Shadle, Wagner and Jacobs, '36) suggested the advisability of investigations of other rodents.

### MATERIAL AND METHOD

There were two groups of experimental animals. Group S contained sixteen females and eight males, of which several were observed and measured for 1 year or more. At the time the measurements were begun on the individuals of group S, they ranged in age from 2 months to 3.5 years, with an average of 5 months. Group SV consisted of eleven females and eleven males, each of which was under observation for 21 to 34 weeks. The observations and measurements on an animal were begun when it was between 2 and 4 months of age. The average age of the group at the beginning of observations was 2.5 months. These forty-six animals were taken at random and do not represent an especially chosen group.

During the winter the animals were fed on a well-rounded grain and hay diet with cod liver oil once or twice a week and green material once to three times a week. From June to September the hay was largely replaced by freshly cut grass. Since the rate at which the incisors usually grow and wear away, was the point to be determined, no special feeding was attempted.

The apparatus and method used in marking the incisors and making measurements, are fully described by Shadle ('36). A zipper tube (Shadle and Skarupinski, '35), which greatly facilitates the process of marking and measuring the teeth, was used in holding the guinea pigs.

The extrusion growth of the incisor, measured in millimeters, represents the length of tooth substance produced and extruded beyond the gingival line. The attrition of the incisor represents the length, in millimeters, of tooth worn away. A V-shaped transverse groove, made on the convex, labial surface of the tooth, served as a mark, from which the weekly attrition measurements to the free cutting edge were made. As the distal end of the tooth wore away, the groove on the anterior surface of the incisor gradually approached the cutting edge, and before it finally disappeared, another groove was made across the tooth.

Throughout the data and the discussion, the measurements are written in millimeters, after the fashion of a dental formula, namely,

$$\begin{array}{l} \text{Upper right incisor} \mid \text{Upper left incisor} \\ \text{Lower right incisor} \mid \text{Lower left incisor} \end{array}, \text{ for example, } \begin{array}{l} 1.6 \mid 1.7 \\ 1.9 \mid 1.9 \end{array} \text{ mm.}$$

are the amounts of attrition of the individual incisors for 1 week.

#### DATA

During a total of 580 weeks, the females of group S showed a total amount of extrusion growth and attrition of  $\begin{array}{l} 990.7 \mid 978.9 \\ 1199.8 \mid 1184.5 \end{array}$  mm. The upper right incisor and the lower right incisor surpassed the corresponding left incisors by  $\begin{array}{l} 11.8 \\ 15.3 \end{array}$  mm. The total amounts of attrition of the upper incisors and of the

lower incisors were, respectively,  $\frac{1969.6}{2384.3}$  mm. There were 414.7 mm. greater extrusive growth and attrition in the lower than in the upper incisors. The average weekly rates of extrusive growth and attrition of the incisors of the females in group S were:  $\frac{1.708}{2.068} | \frac{1.687}{2.042}$  mm.; the right incisors surpassed the corresponding left incisors by  $\frac{0.021}{0.026}$  mm.

In a total of 284.5 weeks, the females of group SV produced and wore away a total of  $\frac{457.6}{560.3} | \frac{465.0}{567.8}$  mm.; the amount of extrusive growth and attrition of the upper left exceeded the upper right incisor by only 7.4 mm., and the lower left exceeded the lower right by only 7.5 mm. The total amounts of extrusive growth and attrition of the upper and lower incisors were, respectively  $\frac{922.6}{1128.1}$  mm., or 205.5 mm. more extrusive growth and attrition in the lower than in the upper incisors. The average weekly rates of extrusive growth and attrition for the females of group SV were  $\frac{1.608}{1.995} | \frac{1.634}{1.969}$  mm.

Combining the results obtained in the two groups of females during 864.5 weeks, the twenty-seven animals wore away  $\frac{1448.3}{1760.1} | \frac{1443.8}{1752.3}$  mm. The upper right incisor surpassed the upper left by 4.5 mm. and the lower right incisor surpassed the lower left by 7.8 mm. The upper incisors had a total attrition of 2892.1 mm. and the lower incisors a total of 3512.4 mm., or 620.3 mm. more than the uppers. The average weekly rates of extrusive growth and attrition of these females were:  $\frac{1.676}{2.037} | \frac{1.670}{2.028}$  mm.; the computed annual amounts of attrition were:  $\frac{87.15}{105.92} | \frac{86.84}{105.45}$  mm.

During a total of 292 weeks, males belonging to group S had total amounts of attrition of  $\frac{572.5}{706.2} | \frac{571.9}{691.8}$  mm. The upper right incisor exceeded the upper left one by only 0.6 mm. and the lower right incisor exceeded the lower left by 14.4 mm. The combined total amounts of the two upper and the two lower incisors were, respectively,  $\frac{1144.4}{1398.0}$  mm., or 253.6 mm. greater attrition of the lower teeth. The average weekly extrusive growth and attrition rates of these incisors were:  $\frac{1.960}{2.418} | \frac{1.058}{2.369}$  mm.

In 301.5 weeks of observation, the amounts of extrusive growth and attrition for the males of group SV were:  $\frac{555.5}{732.2} | \frac{558.3}{737.5}$

mm. The two upper incisors totaled 1113.8 mm. and the two lower incisors totaled 1469.7 mm. The lowers exceeded the uppers by 355.9 mm. in extrusive growth and attrition. The average weekly rates of the incisors of these animals were  $\frac{1.842}{2.428} | \frac{1.851}{2.446}$  mm.

During 593.5 weeks, the total amounts of extrusive growth and attrition of the male guinea pigs were:  $\frac{1128.0}{1438.4} | \frac{1130.2}{1429.3}$  mm. The upper right incisor exceeded the upper left by only 2.2 mm. and the lower right incisor surpassed the lower left by 9.1 mm. The upper incisors had a total attrition of 2258.2 mm., and the lower incisors, a total of 2867.7 mm., or 609.5 mm. more than the uppers. The average weekly rates of extrusive growth and attrition of these males were:  $\frac{1.900}{2.423} | \frac{1.904}{2.408}$  mm.; the computed annual amounts were  $\frac{98.8}{125.9} | \frac{99.0}{125.2}$  mm.

The total amounts of extrusive growth and attrition of the forty-six animals during 1458 weeks, or 28 years and 2 weeks, were  $\frac{2576.3}{3198.5} | \frac{2574.0}{3181.6}$  mm. The upper right exceeded the upper left by only 2.3 mm. and the lower right exceeded the lower left by only 16.9 mm. The total amounts of attrition of the upper incisors and the lower incisors were, respectively,  $\frac{5150.3}{6380.1}$  mm., or 1229.8 mm. more extrusive growth and attrition in the lowers than in the uppers.

#### DISCUSSION

An outstanding point in the study of the guinea pig is the greater amount of extrusive growth and attrition of the males, as compared with that of the females. The upper incisors of the males annually produce and wear away approximately 14% greater length of tooth than the upper incisors of the females. The lower incisors of the males wear away, annually, approximately 19% greater length of tooth than the lower incisors of the females. An important factor in accounting for this marked sexual difference in the amounts of extrusive growth and attrition, is the behavior of the guinea pigs in chattering their teeth. Although this reaction is shown by both sexes, it is particularly striking in the males during anger, fear, and sexual excitement. The female domestic



rabbits (Shadle, '36) showed much greater attrition than the male rabbits. The female albino rats (Shadle, Wagner and Jacobs, '36) also surpassed the males in extrusive growth and attrition, but by a narrower margin.

The average weekly rates and the annual amounts of extrusive growth and attrition of the lower incisors in the guinea pigs are markedly higher than those of the upper incisors. This finding is in accord with the condition in domestic rabbits and also in albino rats.

In the guinea pigs, the right incisors very frequently slightly exceed the left incisor in extrusive growth and attrition, however, the difference is usually so slight that it is of no significance. In albino rats the right and left incisors of a pair show a greater difference in the attrition rate. The rabbits exhibit a very marked difference between the right and left of a pair of incisors.

Dalldorf and Zall ('30) give measurements for their guinea pigs but because of the difference in the methods and in the objective of the study little ground is presented for comparison of results. Their experiments were made with animals on special diets and these experiments were made with an ordinary balanced daily ration. The fact that they measured only one of the lower teeth, offers another difficulty in comparison of data, because the uppers and lowers have markedly different rates of extrusive growth and attrition. The removal, every fifth day, of the exposed portion of the teeth which Dalldorf and Zall were measuring, probably had an effect of increasing the rate of growth of these teeth. This suggestion is based upon some unpublished data of the senior author, in which breaking of the lower incisor near the gingival line, was followed by a rapid rise in the weekly rate of extrusive growth. In these cases, the abrupt increase to three or four times the average weekly growth, gradually declines in succeeding weeks to the usual rate. The sexual differentiation in the rates of growth was not determined in the animals used by Dalldorf and Zall. This serves as an obstacle to a comparison with our results.

## CONCLUSIONS

1. The forty-six guinea pigs, nineteen males and twenty-seven females, during an accumulation of 1458 weeks (28 years and 2 weeks), exhibited the following average weekly extrusive growth and attrition rates, and annual incisor tooth production:

Male:

Weekly rate,  $\frac{1.900}{2.423} | \frac{1.904}{2.408}$  mm.; computed annual production  $\frac{98.80}{125.90} | \frac{99.00}{125.20}$  mm.,

or approximately, 4 inches in the uppers, and 5 inches in the lowers.

Female:

Weekly rate,  $\frac{1.676}{2.037} | \frac{1.670}{2.038}$  mm.; computed annual production  $\frac{87.15}{105.92} | \frac{86.84}{108.45}$  mm.,

or approximately, 3.5 inches in the uppers, and 4.25 inches in the lowers.

2. Adult male guinea pigs have greater average weekly extrusive growth and attrition than the females.

3. The behavior during anger, fear and sexual excitement, is an important factor in causing this greater attrition in the male guinea pig.

4. The lower incisors produce and wear away greater length of tooth and a greater amount of tooth substance than the upper incisors.

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## AN IMPROVED FUSED QUARTZ LIVING TISSUE ILLUMINATOR <sup>1</sup>

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TWO FIGURES

The fused quartz rod method of illuminating living tissues has been described in detail in two previous articles (Knisely, '36, '37).<sup>2</sup> By means of this method many of the internal organs of small living animals can be studied with the microscope while they are in their normal locations and with their blood vessels, nerves and attachments intact.

### SUMMARY OF THE METHOD ALREADY REPORTED

Briefly, the method depends upon two principles: First, the conduction of light to the tissue through a fused quartz rod.<sup>3</sup> This is so shaped that it exerts but little pressure on the organs between which it passes, and little, or, in the best experiments, no tension or pressure on the structures to be

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This illuminator was demonstrated on August 27, 1936, during the general scientific meeting of the Marine Biological Laboratory, Woods Hole, Mass.

<sup>2</sup> The 1937 article has the better discussion of the problem of controlling the temperature of illuminated tissues.

<sup>3</sup> Barta ('35) has devised an illuminator which consists of a short glass rod having one end drawn out to a very sharp point. This point is thrust from below upward, or from other angles, right into the tissue which is directly under the microscope objective. For an example of the use of the method see Barta's ('36) study of the living frog heart.

studied. Second, the maintenance of the normal temperature of the illuminated area by continuously removing as fast as it is produced, the heat developed there by transformation of light energy. When intense illumination is employed, this heat is removed by means of a gently flowing stream of a physiological solution (such as Ringer's) which passes between the tip of the rod and the illuminated tissue. Figure 1 shows the delivery tip of the simplest of the original models.

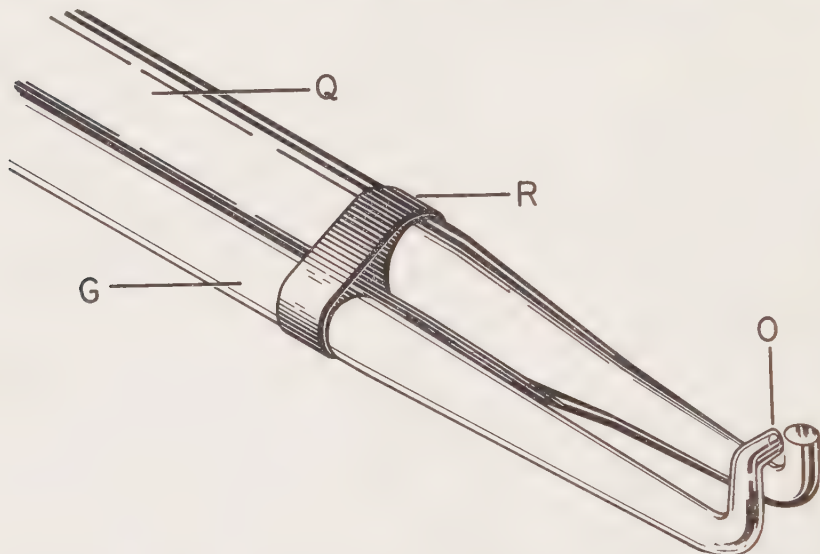


Fig. 1 The delivery tip of the simplest type of fused quartz living tissue illuminator. O, orifice of glass tube (G) which is fastened to fused quartz rod (Q) by small rubber band (R). The tube can be any shape as long as it is adjusted to direct its solution between the tip of the rod and the overlying tissue.

The position of the glass tube which conducts the solution must be carefully adjusted so that the orifice directs a gentle flow across the tip of the fused quartz rod. This model, although inexpensive, easily made, and a very efficient illuminator when the direction of the tube's orifice is well adjusted, has two disadvantages: 1) it requires frequent attention during an experiment to insure that the tip of the tube does not slip away from that of the rod, and 2) the two parts occupy more space than is readily available in some locations.

## THE IMPROVEMENT IN THE METHOD

Figure 2 shows the principle of an illuminator which overcomes the first, and minimizes the second, of the disadvantages mentioned by delivering both light and fluid to the spot to be studied through a single fused quartz unit. The left end, the base (b) of which receives the light, is a solid rod to which have been fused two lengths of fused quartz tube, a longer one (shown here with a light-concentrating segment and delivery tip) through which both light and fluid are delivered to the tissue, and a short side tube to receive the fluid.

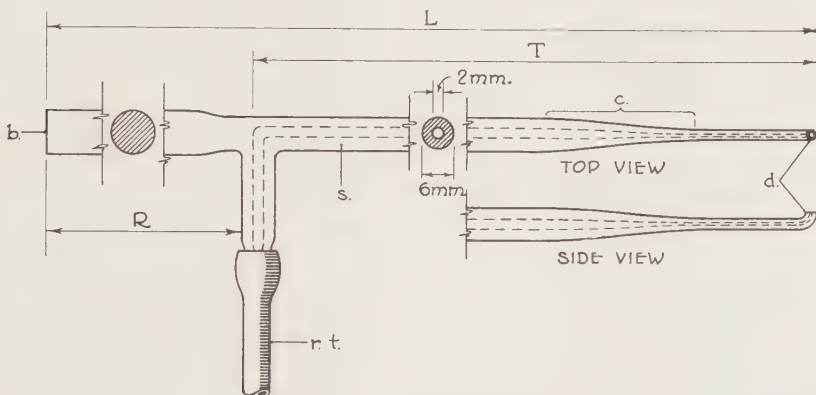


Fig. 2 An improved illuminator. The solution is conducted through the tubular tip of the rod and thus cannot fail to go to the exact spot illuminated. The elimination of the accompanying glass tube permits instruments of this type to be inserted into very small spaces. L, the over-all length; R, the rod; T, the tube; b, base which receives light; r.t., rubber tube supplying solution; c, light-concentrating segment; d, delivery tip.

In making the instrument the desired delivery tip is first drawn on a length of tube, and the tube fused to the solid rod. Then, using a fine needle-pointed flame, a small hole is blown in the tube just at the point where it joins the rod, and a short side tube fused on over the hole. The surface where the three parts unite must be smooth and wrinkle-free to minimize the loss of light at this point.<sup>4</sup>

<sup>4</sup> Instruments of this type (except the bending and grinding of the delivery tips) have been made for me by James Graham, University of Pennsylvania Medical School, Philadelphia; H. Struers, Chemiske Laboratorium, Skindergade 38, Copenhagen, Denmark; and by C. C. Van Hespén, Department of Chemistry, The University of Chicago. The delivery tips I have shaped myself, in order to fit them to the problem at hand.



Thus far the tubing used in making these illuminators has had its external and internal diameters in the ratio of about 3:1. (Tubing with 7 mm. external and 2 mm. internal diameter has given good results.) This makes the cross section area of the tube's light-conducting wall roughly eight-ninths of its total cross section area. Because the lumen occupies such a small fraction (one-ninth) of the total cross section area, and because the solution in it conducts considerable light, a good instrument of this type illuminates tissues almost as efficiently as a solid rod of the same size. The tissue directly over the orifice of the tip is not as brightly illuminated as that over the fused quartz portion. This may cause difficulty in photography, but in ordinary use it has not proved to be significantly disadvantageous.

One difficult part of making these instruments is drawing and bending the delivery tips, and considerable practice may be required before one can make tips which give the brightest illumination. It is, of course, important that the lumen and orifice of the tips are not large enough to reduce the light-conducting efficiency of the instrument. At the same time it is important that the orifice of the delivery tip be large enough so that the rate of flow of solution required to carry away the heat developed can be maintained with very little pressure. If the orifice were too small an adequate rate of flow could be maintained only at a high pressure and the force of a stream would compress delicate tissue or the beating of a stream would initiate a hyperemia.

In general it is wise to have the tube (T) long and the rod (R) short when the instrument is made, so that when a tip is broken a new one can be drawn on the tube and a corresponding length of rod fused to the other end if desired. However, for mammalian work it is best to have the rod long and tube short so that the warm solution has little time to cool while traversing it.

Illuminators of this type are now being used instead of the length of rod previously shown extending between the light filter and the specimen (fig. 5, Knisely, '36; fig. 1, Knisely, '37). Their over-all length (L, fig. 2) is from 25 to 35 cm.

Their delivery tips are fire-polished or have a 'ground glass' surface prepared as described by Knisely ('37). The tip is from 1.5 to 2.5 mm. in diameter with an orifice one-third or a little less than one-third as great. Instruments using these principles can of course be made with various dimensions and with either the rod or tube, or both, curved or bent to suit them for particular purposes.

For aseptic techniques, such instruments can be steam-sterilized, flamed (fused quartz can be flamed when wet), or washed in the usual bactericidal solutions.

#### SUMMARY

The described improvement in fused quartz illuminators consists in providing the illuminating rod with a hollow tip. Through this tip both light and the solution which carries away the heat developed by transformation of light energy are delivered to the structure to be studied. A side tube receives the solution. Advantages are, first, that the solution cannot fail to go to the illuminated area, and second, that the elimination of the accompanying glass tube permits an instrument of this type to be made so small that, even when inserted into very narrow places, it exerts little or no pressure on structures between which it passes.

The light-conducting efficiency of this type of illuminator depends upon several factors. As with the original rod, the material must be transparent and have a smooth outer surface. Secondly, the tubing used for making the hollow tip must have its external and internal diameters in such a ratio (3:1 has been found effective) that the light-conducting wall has a much greater cross sectional area than the solution-conducting lumen. Finally, the surface at the point of fusion of rod, side tube, and hollow delivery tip must be smooth.

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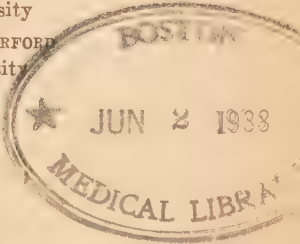
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MAY 25, 1938

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# SUPPLEMENT



# PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

## FIFTY-FOURTH SESSION

*University of Pittsburgh, Pittsburgh, Pa.,*

*April 14, 15 and 16, 1938*

THURSDAY, APRIL 14, 1938

10.00 A.M. The Fifty-fourth Session of the American Association of Anatomists was called to order by President Frederic T. Lewis. The president announced the appointment of the following committees:

*Auditing Committee:* Prof. S. I. Kornhauser, Chairman; Prof. Eben J. Carey.

*Nominating Committee for 1939:* Prof. George L. Streeter, Chairman; Prof. Basil C. H. Harvey, Prof. Philip E. Smith.

The rest of the session was devoted to the scientific program.

FRIDAY, APRIL 15, 1938

12.30 P.M. Business meeting, President Frederic T. Lewis in the chair. The Secretary-Treasurer reported that the minutes of the Fifty-third Session were printed in The Anatomical Record, vol. 68, no. 1 (supplement), pp. 1-41, inclusive. On motion, seconded and carried, the minutes were approved as printed.

*Report of the Treasurer:* The Secretary-Treasurer made the following report for the year 1937:

|                                                                                                                                                                                     |           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| Balance on hand in checking account, December 31, 1936,                                                                                                                             |           |
| last audit of accounts .....                                                                                                                                                        | \$672.40  |
| Receipts from dues and abstracts .....                                                                                                                                              | 727.52    |
| <i>Total credits</i> .....                                                                                                                                                          | \$1399.92 |
| Expenditures, 1937:                                                                                                                                                                 |           |
| Postage, stationary, supplies .....                                                                                                                                                 | 80.16     |
| Printing .....                                                                                                                                                                      | 58.18     |
| Programs, 53rd meeting .....                                                                                                                                                        | 109.00    |
| Secretarial assistance .....                                                                                                                                                        | 0.80      |
| Traveling expenses, secretary .....                                                                                                                                                 | 23.70     |
| Identification badges .....                                                                                                                                                         | 21.00     |
| Wistar Institute (abstracts) .....                                                                                                                                                  | 114.00    |
| Local Committee, 53rd meeting .....                                                                                                                                                 | 150.00    |
| Telegrams .....                                                                                                                                                                     | 1.00      |
| Nomenclature report .....                                                                                                                                                           | 32.80     |
| Lincoln-Alliance Bank (collection fees, etc.) .....                                                                                                                                 | 0.75      |
| <i>Total expenditures</i> .....                                                                                                                                                     | 591.39    |
| Balance on hand December 31, 1937, and deposited in the name<br>of the American Association of Anatomists in the Lincoln-<br>Alliance Bank and Trust Company, Rochester, N. Y. .... | 808.53    |
| Special interest account no. 19439, Lincoln-Alliance Bank                                                                                                                           |           |
| Balance December 31, 1936 .....                                                                                                                                                     | 646.02    |
| Interest .....                                                                                                                                                                      | 12.98     |
| Balance December 31, 1937 .....                                                                                                                                                     | 659.00    |
| <i>Total balance</i> .....                                                                                                                                                          | \$1467.53 |

It will be noted that the checking account gained \$136.13, and the special account gained \$12.98, making a total gain for the year of \$149.11.

GEORGE W. CORNER, *Treasurer*.

*Report of the Auditing Committee:* Professor Kornhauser. reported as follows:

"The undersigned Auditing Committee has examined the accounts of the Secretary-Treasurer for the year 1937, and finds total receipts reported to be those entered in the journal, expenditures in accordance with receipted bills, and the balance on hand December 31, 1937, in agreement with the bank statement of that date and pass-book of special account."

(Signed) S. I. KORNHAUSER,  
EBEN J. CAREY.



On motion, duly seconded, the reports of the Secretary-Treasurer and of the Auditing Committee were accepted and adopted.

*Election of Officers:* Prof. R. R. Bensley, Chairman of the Committee on Nominations for 1938, placed the following nominations before the Association:

For President, Stephen W. Ranson; for First Vice-President, T. Wingate Todd; for Second Vice-President, Albert Kuntz; for Secretary-Treasurer, Eliot R. Clark; for Members of the Executive Committee, George W. Corner, The University of Rochester; Olof Larsell, University of Oregon.

On motion, seconded and carried, the Secretary was instructed to cast a ballot for the election of the above-named, with terms of office as specified in the Constitution of the Association.

*Election of Representatives:* The Secretary then read, on behalf of the Executive Committee, the following nominations: Representative on the Commission for Standardization of Biological Stains, Professor H. E. Jordan; Representatives on the Council of the American Association for the Advancement of Science, Profs. Harvey E. Jordan and Charles C. Macklin; Delegate to the Union of Biological Societies, Prof. Stacy R. Guild.

The persons proposed were duly elected, with terms as specified in the orders of the Association.

*New Members:* The Secretary presented the following names of persons recommended by the Executive Committee for election to membership in the Association:

APGAR, BESSIE D., Ph.D., *Department of Anatomy, Cornell University Medical College, 1300 York Ave., New York City.*

APGAR, CHARLES S., JR., Ph.D., *Instructor in Anatomy, Cornell University Medical College, 1300 York Ave., New York City.*

BAILLIF, R. N., Ph.D., *Instructor in Anatomy, Louisiana State University, Medical Center, New Orleans, La.*

BUCHANAN, A. R., M.D., *Associate in Neurology, Northwestern University Medical School, Chicago, Ill.*

CHASE, RALPH E., A.M., *Instructor in Anatomy, Oklahoma University Medical School, Oklahoma City, Okla.*

CONWAY, ELEANOR A., Ph.D., *Assistant in Anatomy The University of Chicago, Chicago, Ill.*

- CORBIN, KENDALL B., M.D., Associate Professor of Anatomy, *University of Tennessee, Memphis, Tenn.*
- COUNT, EARL W., Ph.D., Assistant Professor of Anatomy, *New York Medical College, 450 East 64th St., New York City.*
- CURWEN, ALICE O., Ph.D., Assistant Professor of Anatomy, *Woman's Medical College of Pennsylvania, Philadelphia, Pa.*
- CUTULY, EUGENE, Ph.D., *Cleveland Clinic, 93rd and Euclid Ave., Cleveland, Ohio.*
- DEMPSEY, EDWARD W., Ph.D., Fellow in Anatomy, *Harvard Medical School, Boston, Mass.*
- DERBYSHIRE, ARTHUR J., Ph.D., Assistant Professor of Anatomy, *Wayne University School of Medicine, 1512 St. Antoine St., Detroit, Mich.*
- DORRIS, FRANCES, Ph.D., Research Assistant in Embryology, *Yale University School of Medicine, New Haven, Conn.*
- DUSHANE, GRAHAM P., Ph.D., Instructor in Zoology, *The University of Chicago, Chicago, Ill.*
- EDWARDS, EDWARD A., M.D., Assistant in Anatomy, *Harvard Medical School, Boston, Mass.*
- ELPTMAN, HERBERT, Ph.D., Assistant Professor of Zoology, *Columbia University, College of Physicians and Surgeons, 630 West 168th St., New York City.*
- FORBES, THOMAS R., Ph.D., Assistant in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*
- HAMMOND, WARNER S., Ph.D., Instructor in Anatomy, *University of North Carolina, Medical School, Chapel Hill, N. C.*
- HOOKE, CHARLES W., Ph.D., Instructor in Anatomy, *Yale University School of Medicine, New Haven, Conn.*
- JANES, RALPH G., Ph.D., Instructor in Anatomy, *Wayne University School of Medicine, 1512 St. Antoine St., Detroit, Mich.*
- JOHNSON, MYRA L., Ph.D., Instructor in Zoology, *Smith College, Northampton, Mass.*
- JONES, DAVID S., Ph.D., Instructor in Anatomy, *Loyola University Medical School, Chicago, Ill.*
- JONES, RUSSELL, Ph.D., Instructor in Anatomy, *Indiana University School of Medicine, Bloomington, Ind.*
- KIRKMAN, HADLEY, Ph.D., Instructor in Anatomy, *Stanford University, Calif.*
- KNISELY, MELVIN H., Ph.D., Assistant Professor of Anatomy, *The University of Chicago, Chicago, Ill.*
- KOZELKA, A. W., Ph.D., *Hospital of St. Anthony de Padua, Chicago, Ill.*
- LOOSLI, CLAYTON G., Ph.D., M.D., *Billings Memorial Hospital, Chicago, Ill.*
- MAGRUDER, SAMUEL R., Ph.D., Assistant in Anatomy, *Cornell Medical College, 1300 York Ave., New York City.*
- NAHM, LAURA J., Ph.D., Professor of Biology, *Junior College of Flat River, Flat River, Mo.*
- OPPENHEIMER, JANE M., Ph.D., National Research Council Fellow in Zoology, *University of Rochester, Rochester, N. Y.*
- PFEIFFER, CARROLL A., Ph.D., Research Assistant, *Department of Anatomy, Yale University School of Medicine, New Haven, Conn.*
- ROGERS, PHILIP V., Ph.D., Assistant Professor of Biology, *Hamilton College, Clinton, N. Y.*

- SALSPURY, C. R., M.D., C.M., Assistant Professor of Anatomy, *Queen's University, Kingston, Ontario, Canada.*
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- SMELSER, GEORGE K., Ph.D., Instructor in Anatomy, *Columbia University, College of Physicians and Surgeons, 630 West 168th St., New York City.*
- SNIDER, RAY S., Ph.D., Instructor in Anatomy, *University of Nebraska, College of Medicine, Omaha, Neb.*
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- SOLNITZKY, OTHMAR, Ph.D., M.D., Professor of Anatomy, *Georgetown University, School of Medicine, Washington, D. C.*
- SUMMERS, FRANCIS M., Ph.D., Instructor in Anatomy, *Cornell Medical College, 1300 York Ave., New York City.*
- TERNI, TULLIO, M.D., Professor of Anatomy, *The University of Padua, Padua, Italy.*
- TURNER, CLARENCE D., Ph.D., Instructor in Zoology, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*
- WASSERMANN, FRIEDRICH, M.D., *Zoller Foundation, The University of Chicago, Chicago, Ill.*
- WEISSENBERG, RICHARD, M.D., Fellow, *The Wistar Institute, 36th St. and Woodland Ave., Philadelphia, Pa.*

On motion, duly seconded, the Secretary was instructed to cast a ballot for all candidates proposed by the Executive Committee.

*Members deceased:* The Secretary then presented the following statement of the Executive Committee:

*The Association has suffered great loss by the death, during the past year, of seven members*

JOSEPH A. BLAKE, born August 31, 1864, died August 12, 1937. Elected to membership in 1896. A distinguished surgeon, formerly Professor of Surgery in the College of Physicians and Surgeons, Columbia University, New York.

HENRY HOMER COLLINS, born December 10, 1885, died August 31, 1937. Elected to membership in 1927. Professor of Biology, the University of Pittsburgh. Investigator of growth and regeneration.

WILLIAM M. CONANT, born 1856, died February 18, 1937. His name appeared in the first published list of members, 1890. Honorary Consulting Surgeon to the Massachusetts General Hospital.

HENRY H. DONALDSON, born May 12, 1857, died January 23, 1938. Elected to membership in 1901. President 1916-1917. Member of The Wistar Institute of Anatomy and Biology. (A resolution on the death of Doctor Donaldson is printed in these minutes.)

MILTON J. GREENMAN, born June 14, 1866, died April 7, 1937. Elected to membership in 1903. Director of The Wistar Institute of Anatomy and Biology. (A resolution on the death of Doctor Greenman is printed in these minutes.)

HUGH D. REED, born March 4, 1875, died August 20, 1937. Elected to membership in 1910. Professor of Zoology, Cornell University.

HARRIS E. SANTEE, born October 15, 1864, died February 28, 1936. Elected to membership in 1905. Physician in Chicago. At various times professor in several medical schools.

*Members resigned:* The Secretary then announced the resignation of the following members:

RICHARD HUNTER, Belfast, Ireland  
G. W. McNUTT, Seattle, Washington  
P. POTTER, Butte, Montana

*Members dropped:* The Secretary then announced the following names dropped from the list of members on account of non-payment of dues for the past 2 years:

E. BARTA, Kisvarda, Hungary  
J. S. BAXTER, Cambridge, England  
C. FISHER, Northwestern University  
C. W. HAZEN, Richmond, Va.  
P. L. MULL, University of Mississippi

*Payment to Local Committee:* In accordance with the policy adopted by the Association at the Forty-third Session, it was moved, seconded and voted that the Treasurer be authorized to pay the sum of \$150.00 from the general funds of the Association to the local committee on arrangements at the University of Pittsburgh, to aid in defraying the cost of entertainment of the Association during the present meeting.

*Dues for the year 1938:* A recommendation of the Executive Committee, that dues for the year 1938 be set at one dollar per member, was adopted by the Association.

*Place of next meeting:* It was announced on behalf of the Executive Committee that the next meeting will be held at Harvard Medical School, Boston, Massachusetts, April 6, 7 and 8, 1939.

*International Congress:* In response to an invitation from the Anatomical Society of Great Britain and Ireland to take part in an International Anatomical Congress to be held in England in 1940, it was unanimously voted to take part.

*The Anniversary Medal:* At the request of President Lewis, Professor Addison spoke briefly in explanation of the Fiftieth Anniversary Medal bearing the portrait of the first president of the Association, Joseph Leidy. The medallist, the distinguished sculptor of Philadelphia, Dr. R. Tait McKenzie, is a former member of the Association.

*Overcrowding of the program:* Professor Stockard suggested that the Executive Committee should consider seriously ways and means of reducing the number of papers presented at the meeting. The President stated that the Executive Committee had already voted to appoint a sub-committee for that purpose.

*Resolutions regarding animal experimentation:* At the request of Professor Danforth the Association considered a proposed resolution indicating disapproval of proposed legislation in California which would harmfully limit the use of animals for scientific experimentation. The matter was referred to a committee consisting of Professors Stockard and H. M. Evans and the Secretary.

*Resolutions:* A special Committee consisting of Professors Conklin, Hardesty and Addison, presented the following resolution, which was unanimously adopted:

Henry Herbert Donaldson died on January 23, 1938, in his eighty-first year, after a very short illness. He was born in Yonkers, N. Y., May 12, 1857 and received his early education at Phillips Academy, Andover and at Yale College. On graduating from Yale in 1879 he spent a year in the Scientific School, where with R. H. Chittenden he did his first piece of research, and clearly showed evidence of his unusual mental capacities. After a year as a medical student at the College of Physicians and Surgeons, New York, he was offered and accepted a fellowship in biology under H. Newell Martin at Johns Hopkins University and there entered upon his long career of investigative activity. He studied abroad with von Gudden, Forel and Meynert. He became Assistant Professor of Neurology in Clark University, from which position he went to the Professorship of Neurology at the newly established University of Chicago. There he wrote his book, "The Growth of the Brain," and outlined the field to which he devoted himself for the rest of his life. In 1906 Doctor Donaldson became the first director of Research and Professor of Neurology at The Wistar Institute of Anatomy, where on a large colony of experimental animals, albino rats, he and his associates worked out under controlled conditions many problems concerning the growth of the nervous system and of other parts of the body. The results of these studies were the basis of the book "The Rat—Data and Reference Tables," first edition



1915, second edition 1924, and of over 300 separate papers. Owing to Doctor Donaldson's activities in Chicago and later in Philadelphia the albino rat has become the most widely used laboratory mammal.

Doctor Donaldson was President of the American Association of Anatomists in 1916-1917. In his presidential address he said, "Were I asked to name some direction in which we might extend our work I should naturally lay weight on post-natal growth in the terms of cell multiplication and cell structure, with its many subsidiary problems." This is the keynote to his scientific career. He had a well-defined plan to which he adhered: many topics were considered but all had a bearing upon the main subject, that of growth.

Doctor Donaldson was a member of the original Advisory Board of The Wistar Institute, a member of the National Academy of Sciences, Vice-President of the American Philosophical Society and a past President of the American Neurological Association.

He received the degree of Ph.D. at Johns Hopkins University in 1885 and the honorary Sc.D. from Yale in 1906 and from Clark in 1937. On his eightieth birthday, a bronze portrait medallion of Doctor Donaldson was presented to him by the University of Pennsylvania Lenape Club, of which he had been President for twenty years.

To his colleagues Doctor Donaldson was known as a man of infinite patience, evenness of temper and nobility of character.

By this resolution the American Association of Anatomists records its appreciation of the life and services of Doctor Donaldson and expresses its sorrow at the loss of a charming and helpful associate.

A special committee, consisting of Professors Boyden, Herrick, Stockard and McClung, presented the following resolution, which was unanimously adopted:

The American Association of Anatomists wishes to express a deep sense of loss in the death of Milton J. Greenman on April 7, 1937. Doctor Greenman became a member of the Association in 1903 and served American anatomy in a very active way from that time until his death. In 1905 he was appointed Director of The Wistar Institute and he immediately organized a Scientific Advisory Board for the guidance of the Institute. The functioning of this Board and the maintenance of The Wistar Institute as an independent institute of anatomy have been unique. Doctor Greenman not only developed a scientific staff at the Institute, which before his time had been only a museum, but he soon became convinced that the Institute could render a great service in aiding the anatomical journals and publications of this country. Thirty years ago, in 1908, five morphological periodicals of national importance were brought together under one publishing management at The Wistar Institute. Prior to this time the existence of the journals had depended upon the efforts of private individuals, and one of them, the *Journal of Morphology*, had ceased publication in 1903. The ownership of the journals remained for some time in the hands of the *Journal Trust* and the Editorial Boards, but all have now been turned over as property of The Wistar Institute. The Editors fully appreciate the many difficult problems which Doctor Greenman solved in carrying on the publication and circulation of

the journals through times of world war and financial stress. Milton Greenman was a dynamic personality who worked with untiring energy for the accomplishment of major purposes—the welfare of The Wistar Institute, as he saw it, and the promotion of anatomical publications in this country. His own name was not placed forward and his efforts were never directed toward selfish ends.

The business session then adjourned.

### SATURDAY, APRIL 16, 1938

12.30 P.M. A business session followed the scientific sessions of the morning.

*Resolution concerning animal experimentation:* The committee appointed at the business session of April 15th offered the following resolution, which was unanimously adopted and ordered to be forwarded to Professor Danforth:

Because the maintenance of the public health, the protection of our children from disease, their growth and nutrition, and the treatment of illness in maturity and old age all depend upon the progress of medical and biological science, the American Association of Anatomists believes that any attempt to prevent or limit the proper use of animals in the investigation of scientific problems is a threat to the welfare of mankind. Instead of impeding progress by legislative enactment, every effort should be made to facilitate the advancement of those sciences upon which our health and well-being so largely depend.

*Resolution of appreciation:* The following resolution, proposed by Professor Boyden, was unanimously adopted:

The American Association of Anatomists, in session at Pittsburgh, April 14, 15 and 16, 1938, wishes to acknowledge its great indebtedness to Chancellor Bowman and to the governing boards of the University of Pittsburgh and the Mellon Institute, and to the Local Committee of the Department of Anatomy, Doctors Donaldson, Hooker and Hogg, for the gracious and effective arrangements that have provided so memorable an anniversary.

The Association expresses cordial thanks to the Department of Buildings and Grounds of the University, which made it possible to hold our sessions in the great Cathedral of Learning; to the secretarial staff of the Department of Anatomy for unfailing and courteous assistance; and to the student body of the School of Medicine, whose volunteer aid contributed so much to the smooth progress of our sessions.

There being no further business, the Fifty-fourth Session was concluded, in the afternoon, in a joint scientific session with the American Association of Physical Anthropologists.

## MESSAGE FROM THE PRESIDENT OF THE UNITED STATES

At the Fiftieth Anniversary Dinner on Friday, April 15th, the President of the Association, Dr. Frederic T. Lewis, read the following message received from the President of the United States:

The White House  
Washington

"To the American Association of Anatomists I wish to convey my warm congratulations on the completion of fifty years of activity as a scientific body.

"Perhaps yours, more than any other scientific discipline, combines the rigors of an exact science with the latitude of a biological science. Anatomy, including comparative anatomy, forms the framework on which numerous other sciences are based. Though many of your number have been active in fields which a few decades ago may not have been thought of, by some, as explicitly anatomical, you have maintained cohesion, and in your ranks are numbered leaders in anatomical knowledge and discovery.

"We continually and vitally need your help in maintaining and increasing the body of anatomical knowledge available, and the American public will be the gainer if your organization will keep intact this history of service."

(Signed) FRANKLIN D. ROOSEVELT.

It was announced that the following reply, prepared by Doctor Lewis, had been forwarded to the White House.

"The President  
The White House  
Washington, D. C.  
Mr. President:

"Stirred by your message of encouragement and congratulations, The American Association of Anatomists, celebrating its Fiftieth Anniversary in Pittsburgh's Cathedral of Learning, will renew its efforts to meet its biological problems. *Multum adhuc restat operis, multumque restabit.*

"In cordial response to your interest in our welfare."

For the Association

GEORGE W. CORNER, *Secretary.*

## ROUND TABLE DISCUSSIONS

*The endocrine control of female sexual and reproductive functions in monkeys and other primates.* An attempt to cover hormonal coordination of genital organs in sequence as in life. Chairman, Edgar Allen. Participants: Edgar Allen, G. W. Bartelmez, J. T. Bradbury, George W. Corner, G. H. Daron, J. H. Elder, E. T. Engle, W. U. Gardner, C. G. Hartman, F. L. Hisaw, J. E. Markee, W. O. Nelson, G. van Wagenen.

The female monkey has been studied extensively because she has menstrual cycles like woman and can be used freely as an experimental animal. Many papers have been presented at previous meetings of the Association on definite, restricted phases of reproduction and associated endocrines. The present Round Table attempted to synthesize outstanding points in the sequence in which they occur in life.

Contributors were asked to submit several subjects (with topical outlines) which they might present briefly (3 or 4 minutes each). These were oriented in a 'time table' which served as agenda, and which was mimeographed and circulated beforehand. Thirty-eight subjects ranging through nine major events in reproductive life traced incidents in endocrine control from 1) the acceleration of growth of genital organs leading to adolescence, through 2) early menstrual cycles without ovulation, 3) ovulation, 4) ovulatory menstrual cycles, 5) mating reactions 6) pregnancy, 7) birth, 8) lactation, and 9) menopause. The objective was to summarize briefly and coordinate—a bird's eye view of the endocrines involved in a complete sexual and reproductive life span.

Speakers carried through as planned and gave effective summaries. Thirty-one of the thirty-eight subjects were presented—the monkey was carried to the end of her first pregnancy. Two brief intermissions allowed some recuperation. There was not adequate time for discussion. A speaker, although allowed three 4-minute periods, sacrificed the continuity he would have had in presenting his work at another session. To be entirely successful such an effort would require a rehearsal! The session proved interesting as an experiment in summarizing and coordinating a great deal of interesting morphological and experimental work, but such a symposium is not recommended for frequent use.

*The lymphocyte.* Chairman, Hal Downey. Participants: William E. Bloom, Warren H. Lewis, Bruce K. Wiseman, Charles A. Doan, Maurice N. Richter, Arthur Kirschbaum.

William Bloom opened the discussion with a splendidly illustrated discussion of the formation of lymphocytes, their morphological criteria and developmental potencies. Experiments with local infections with *B. monocytogenes* showed that regeneration of lymphocytes is largely homoplastic by mitosis of previously existing lymphocytes. However, some transformation of cells of the reticulum to lymphocytes also occurs. Lymphoblasts and immature lymphocytes of the clinical hematologist are not recognized, for it was found that all types of lymphocytes can multiply, especially the larger ones. All are immature in the sense that they can transform to dye-storing macrophages, monocytes, and even granular leukocytes. Variations of mitochondria, basophilia of cytoplasm, etc., are functional variations not concerned with the degree of maturity of the cells.

Variations in nuclear pattern are likewise of little importance for classification of lymphocytes or of the lymphoid stem cells of the clinical hematologists (lymphoblasts, myeloblasts).

In hyperplastic conditions of lymphatic organs new nodules may originate from localized proliferations of lymphocytes in no way related to changes in the reticulum or vascular pattern. Colored photographs showed the migration from vessels to tissue injected with colloidal dye. The transformation of these lymphocytes to dye-storing histiocytes in the tissue removed at different time intervals could be followed in detail. This was an important demonstration for the reason that Aschoff and some other pathologists still do not believe that lymphocytes can transform to the histiocytic type of cell.

Warren H. Lewis discussed lymphocytes and monocytes as seen in tissue culture. The evidence from his cultures seemed to indicate that lymphocytes and monocytes are independent cell types, and that a lymphocyte never transforms to a monocyte. Monocytes survive longer than lymphocytes and may multiply in the cultures. The origin of the monocytes in the lymph node transplants was not discussed. The results are contrary to those of Bloom based primarily on experiments with *B. monocytogenes* in rabbits.

B. K. Wiseman illustrated and discussed the types of lymphoid hyperplasia which may occur in human clinical cases. The degree of maturation which occurs simultaneously with multiplication is of importance for the resulting hematological and clinical picture. Leukemia occurs when the proliferation of immature cells is accompanied by defective maturation. This view is based on the assumption that immature cells of leukemic type are present in normal human lymphatic tissues. This paper was supplemented by remarks along the same line by C. A. Doan.

Papers dealing with the lymphocytes of leukemic mice were presented by Maurice N. Richter and Arthur Kirschbaum. The former brought out the similarities and differences between leukemic lymphocytes and tumor cells in mice. In most respects the cells are similar, but leukemic cells enter the circulation more readily and become more widely disseminated than do tumor cells. Transformation of lymphosarcoma to leukemia and vice versa occurs spontaneously and experimentally. Leukemic lymphocytes originate in definite foci and infiltrate normal tissues. This is contrary to the generally accepted view that lymphatic leukemia originates as a systemic hyperplasia of lymphatic tissue. Genetic factors are of supreme importance in both the spontaneous origin of mouse leukemia and its transmission by means of living leukemic cells. The introduced cells survive and undergo leukemic proliferation crowding out the normal lymphocytes.

A. Kirschbaum, reporting on the work that he has been doing in collaboration with F. J. Lits, L. C. Strong and W. U. Gardner, stated that no one type of lymphocyte of lymphatic leukemia can be regarded as *the* malignant lymphocyte which can produce generalized leukemia or lymphatic tumors. In certain strains of mice the immature leukemic cells ripen to mature cells, and the leukemic proliferation may begin in the reticulum. Lymphocytes and their progenitors may, therefore, become malignant (leukemic or tumor-forming) at any stage of their maturation. Of special interest was his statement that immature lymphocytes morphologically identical with the most immature lymphocytes of leukemia may be



present in small number in the lymphatic tissues of normal mice. In leukemia these immature cells become very numerous, but morphologically they may remain identical with the few immature cells of the normal tissues.

Subcutaneous injection of minute amounts of colchicine caused the temporary regression of lymphoid tumors and lengthened the life of the mice as compared to control tumor mice. Kirschbaum and his co-workers also noted the transition from lymphoid tumor to leukemia described by Richter.

Discussion of the papers of the symposium was lively, and prolonged to such an extent that the session lasted for a little more than 4 hours. Wiseman questioned the identity of the mouse leukemia with the human disease and the opposite view was defended by Kirschbaum. When questioned William Bloom stated that he did not believe in the justification of the division of lymphocytes into immature, young and old forms on the basis of nuclear pattern, basophilia of cytoplasm, etc., for they can all transform to other types of cells. Developmental potentialities are, therefore, of greater importance than morphological characters for the determination of the degree of maturity of lymphoid cells.

All the papers were well illustrated with lantern slides and demonstrations, and W. H. Lewis showed a motion picture of the behavior of lymphocytes and monocytes in tissue culture.

While it is not likely that anyone changed his opinions as the result of the discussion and evidence presented, it was generally agreed that the Round Table and accompanying demonstrations gave an unusual opportunity for the presentation of evidence of progress in hematological research and its interpretation.

*Visceral afferent pathways and visceral sensation.* Chairman, J. C. Hinsey.

Participants: S. W. Ranson, Olof Larsell, J. F. Nonidez, O. R. Langworthy, D. W. Bronk, Kendrick Hare and H. G. Wolff.

In his description of the visceral afferent fibers in cerebrospinal nerves, S. W. Ranson emphasized the fact that their cells of origin are in the dorsal root and cranial ganglia, and that they vary in size from fairly large myelinated to unmyelinated fibers. In support of this, he described experiments on the sensory components of the splanchnic nerves, where degeneration procedures were used to isolate the afferent fibers. He discussed some of the evidence which shows the presence of neurons in the dorsal root and cranial ganglia that may subserve conduction from the C. N. S. to the periphery.

In reviewing the probable function of the receptors in the lung, Olof Larsell suggested that the receptors in the respiratory portion of the lung are chemoreceptors, those in the visceral pleura respond to contact from adjacent lobes, the smooth muscle spindles may be stimulated by changes in tension, and the endings beneath the epithelium of the bronchi are tactile receptors.

J. F. Nonidez described the various types of endings in the heart: aorta, pulmonary artery, the great veins and 'paraganglia,' and discussed their possible functional significance. In hearts of cats, in which all of the postganglionic neurons had been removed and degeneration had occurred, receptors in pulmonary veins were absent, demonstrating origin from dorsal root ganglia of upper thoracic region.

O. R. Langworthy described a submucous plexus of fibers distributed to blood vessels, freely in connective tissue and to deeper layers of the urinary bladder epithelium. This is derived mainly from the sympathetic and, among other things, contains receptors for pain. Myelinated fibers from sacral dorsal root ganglia terminate in arborizations on bladder smooth muscle. These are stretch receptors.

In a consideration of analysis of afferent mechanisms, D. W. Bronk stated advantages in use of action potentials. Activity of receptors is reflected as changes in frequency in their afferent fibers and in numbers of receptors responding. Several questions were posed, among which were: What are structural adaptations of presso-receptors as contrasted to chemo-receptors? What are structural adaptations of some receptors which cause them to show rapid adaptation while others adapt slowly?

Observations on the question of reflex mechanisms within sympathetic chain ganglia were described by Kendrick Hare. Skin galvanic reflex technique and string galvanometer were used to study reflex responses in cats' fore-pads. His observations, on pads in completely deafferented fore limbs and in ones in which preganglionic supply to stellate ganglia had been broken by section of thoracic ventral roots, do not support reflex pathways involving afferent conduction to stellate ganglia from fore limb pads that does not traverse the spinal cord.

H. G. Wolff discussed referred pain, particularly in region of head. He cited cases in which pain produced by distension of temporal artery and of other cranial vessels was referred. Evidence was presented to show that abnormal pulsations of certain cranial vessels caused headache. His slides demonstrated the areas into which pain was referred from pituitary, eyes, teeth, sinuses and posterior fossa. Wolff analyzed several cases of Henry Head and showed that reference into sensory distribution of 5th cranial nerve was probably caused by local involvement rather than by segmental skipping.

*Some aspects of the pelvis and vertebral column.* Chairman, A. W. Meyer, Participants: Gustave B. Schunke, Mildred Trotter, W. E. Chamberlain, William W. Greulich, Walter Sullivan, Adolph Schultz; A. W. Meyer.

The interest shown in the colloquium on the vertebral column and pelvis was gratifying to the participants and heartening to those devoted to gross anatomy. The papers presented were comprised very largely of factual additions to our knowledge of both structure and function.

The observations of Doctor Schunke on the development of the sacro-iliac joints are fundamental and should do much to dispel the confusion that still exists regarding the matter. They revealed the existence of a close parallelism between the development of the cavity in these joints and in the symphysis pubis. Although time did not permit a discussion of the gross nature of the articular surfaces, their frequently interlocking nature was referred to and its bearing on the possible amount of motion stressed.

Professor Trotter's report on the incidence, nature, and relation of supernumerary articular facets in the sacro-iliac region had an important bearing upon the question of motion. The fact that she found some facets covered only by fibrous tissue, others by such tissue containing some cartilage cells, and still others covered by hyaline cartilage, has an important bearing upon the genesis

of these facets. Professor Trotter further called attention to the varying incidence of these facets with age, sex and race and added that attempts to recognize their presence in skiagrams had so far been unsatisfactory.

The range of motion in the pelvic joints, as revealed by skiagrams taken with special techniques, was considered by Professor Chamberlain and threw a revealing light on both the anatomy of and symptomatology in the sacro-iliac region. It also lent an interest to the topic under discussion which it otherwise might not have been thought to possess. It was surprising to learn that Professor Chamberlain has developed a technique which he felt enabled him to recognize so small a vertical displacement at the symphysis as 1 mm.

Professor Greulich's extensive skiagraphic measurements of the female pelvic inlet in primigravid white women and in white nulliparous student nurses, suggest that an old tenet needs reexamination for he found the prevailingly accepted form of female pelvis in only 37% of the latter, and in 45.5% of the former. Professor Greulich further found that labor was less frequently complicated in the unorthodox type of pelvis.

Professor Sullivan referred to the interpretation of vertebral anomalies and to the lack of data regarding variability in the chondrogenous centers as well as to the occurrence of multiple centers in cartilages connected with bone repair.

Professor Schultz's discussion concerned numerical vertebral variation, the relative length of the various regions in anthropoids and man, and the occurrence of a sacro-iliac promontory. He emphasized the fact that there is no direct relation between numerical increase and increase in length of the particular region, or between the erect posture and the presence of a promontory. He further stated that man and the anthropoids differ from the lower catarrhines not only in possessing fewer thoracolumbar and caudal, and more sacral vertebrae, but also by having comparatively larger cervical thoracic and sacral regions and much shorter lumbar regions, and added that among the primates examined, man has the relatively longest cervical and thoracic regions and the comparatively largest lumbar vertebrae, and that reduction of vertebrae in certain regions has progressed farther in some anthropoids than in man.

At the request of the audience the chairman briefly presented illustrations of what he believed represented functional destruction of the intervertebral disks, a phenomenon which is more evident in the lower cervical, the thoracolumbar and especially in the lumbosacral regions. He reported the finding of polished facets upon the coapted surfaces of the bodies of vertebrae, which he believed could only be due to attrition between them after destruction of the intervertebral disks.

In concluding this report, the brevity of which is necessitated by the conditions of publication, the chairman again takes occasion to express his indebtedness to the participants in the colloquium and also to Professors Todd, von Bonin, Grant, and Doctor Stewart for their amplified comments and discussion.

# AMERICAN ASSOCIATION OF ANATOMISTS

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### *Member of the Commission on Standardization of Biological Stains*

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STACY R. GUILD

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- ANDREW, WARREN, M.S., Ph.D., Instructor and Research Fellow in Anatomy, *University of Georgia School of Medicine, Augusta, Ga.*
- ANGULO Y GONZALEZ, ARMANDO W., A.B., B.S., A.M., Ph.D., Associate Member, *The Wistar Institute of Anatomy, Woodland Ave. and 36th St., Philadelphia, Pa.*
- ANSON, BARRY J., A.B., A.M., Ph.D., Associate Professor of Anatomy, *North-western University Medical School, 303 East Chicago Ave., Chicago, Ill.*



- APGAR, BESSIE D., Ph.D., *Department of Anatomy, Cornell University Medical College, 1300 York Ave., New York City.*
- APGAR, CHARLES S., JR., Ph.D., *Instructor in Anatomy, Cornell University Medical College, 1300 York Ave., New York City.*
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- ARMSTRONG, PHILIP BROWNELL, B.S., M.D., *Assistant Professor of Anatomy, Cornell University Medical College, 1300 York Ave., New York City.*
- ASDELL, SYDNEY ARTHUR, M.A., Ph.D., *Professor of Animal Physiology, State College of Agriculture, Cornell University, Ithaca, N. Y.*
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